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I Mini-Simposio de Investigación en Ciencias Experimentales

FESTIVIDAD DE SAN ALBERTO MAGNO

15 DE NOVIEMBRE DE 2011

LUGAR DE CELEBRACIÓN

SALA DE GRADOS Y HALL DEL AULARIO IV

CAMPUS DE LA CAÑADA

CARRETERA DE SACRAMENTO, S/N

04120, ALMERÍA.

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Comité Organizador

Mª del Carmen Cerón García
Ignacio Fernández de las Nieves

Comité Científico Evaluador

Agradecemos a los miembros del Comité Científico Evaluador por su apoyo desinteresado e incondicional en la evaluación de los trabajos presentados.

Francisco García Camacho
Antonia Garrido Frenich
Juan Francisco Mota Poveda
José Antonio Rodríguez Lallena
Antonio Manuel Romerosa Nievas

2. Cartas de Bienvenida

Carta del Decano de la Facultad de Ciencias Experimentales

En nombre de la Facultad de Ciencias Experimentales, quiero expresar la profunda convicción de que la apuesta decidida por la Investigación es uno de los pilares irrenunciables de la actividad universitaria. Hoy, cuando no cesan voces que ponen en cuestión la función social de la Universidad, en general, y de la Investigación Básica, en particular, nuestro centro quiere animaros en esta estimulante tarea que habéis elegido como profesión: la creación y transmisión de Saber desde el ámbito de la Universidad Pública.

Este primer mini-simposio nace como el resultado de la maduración de unas experiencias ya vividas en los años anteriores y que han puesto de manifiesto que el quehacer del día a día de nuestros investigadores es lo suficientemente rico como para ser presentado en un tipo de formato atractivo, a la vez que comprometedor de cara a futuras ediciones.

Por ello, celebrar la festividad de nuestro patrón san Alberto Magno, con una actividad de este tipo, no sólo es ganar en prestancia para nuestras actividades conmemorativas; este mini-simposio es un homenaje a todos los investigadores que lo hacéis realidad. En particular, quiero destacar a los compañeros María del Carmen Cerón, Vicedecana de Investigación e Innovación Tecnológica de la Facultad de Ciencias Experimentales, y a Ignacio Fernández de las Nieves, Director de los Servicios Centrales de Investigación de la UAL, por haber hecho realidad este acontecimiento del que este Libro es testimonio.
Enhorabuena a todos y feliz celebración tengamos de nuestro patrón.

Enrique de Amo Artero
Decano

Carta del Comité Organizador

En nombre del comité organizador, nos complace darles la bienvenida al I Mini-Simposio de Investigación en Ciencias Experimentales, organizado con motivo de la festividad de San Alberto Magno. Nos gustaría además agradecerles su presencia en esta primera edición, que esperemos sea la primera de una serie extensa que con el tiempo se consolide en nuestra institución.

Mediante este I Mini-Simposio se desea fomentar la difusión de la investigación realizada por las nuevas generaciones de investigadores y establecer la cooperación entre científicos de todas las áreas de la ciencia. Su celebración tiene lugar en un año de especial relevancia al conmemorar el centenario del Premio Nobel otorgado a Marie Curie por sus aportes a la Química y de la fundación de la Asociación Internacional de Sociedades Químicas. Es por ello que se trata de un año excepcional con el que comenzar este certamen, presentar nuestros resultados científicos más recientes, y compartir perspectivas y debates de nuestro interés.

Estamos plenamente convencidos de que será un encuentro académico, científico y social muy enriquecedor para todos los asistentes. Les esperamos a todos el día de San Alberto Magno – patrón de Ciencias – y poder decir una de sus frases célebres: *"Yo mismo lo he experimentado"*.

Reciban un cordial saludo,

María del Carmen Cerón
Ignacio Fernández
Comité organizador

3. Patrocinio del Mini-Simposio

Agradecemos a la Universidad de Almería, a la Facultad de Ciencias Experimentales, a los Servicios Centrales de Investigación (SCI), al CAMPUS DE EXCELENCIA AGROALIMENTARIO (CeiA3) y a las empresas DICSA, Scharlab, Albus y Fischer-Scientific el apoyo prestado para la organización de este encuentro.

4. Información importante del Mini-Simposio

En la lucha por una investigación de calidad en España, desde la Facultad de Ciencias Experimentales creemos imprescindible que la comunidad universitaria conozca qué es lo que nuestra labor profesional aporta a la misma. La creación de una auténtica Carrera Investigadora, exige un clima en el que la creación científica sea asumida como un valor en sí mismo, y para ello, los jóvenes investigadores debemos saber hacer llegar a la sociedad la importancia de nuestro trabajo.

Es por esto, que la Facultad de Ciencias Experimentales de la UAL convoca el I Mini-Simposio de Investigación en Ciencias Experimentales.

Tema

El I Mini-Simposio pretende ser un foro de encuentro e intercambio de ideas entre los investigadores de la Facultad de Ciencias Experimentales. Se pretende generar un entorno para además de presentar resultados científicos, ideas y proyectos, se compartan perspectivas y así debatir temas de nuestro interés.
Para ello los investigadores podrán enviar resúmenes de trabajos de investigación que hayan inscrito en congresos nacionales e internacionales durante el curso académico 2010/2011, o bien enviar resúmenes de trabajos aún por diseminar nacional o internacionalmente. Todos aquellos resúmenes admitidos por el Comité Científico serán presentados en forma de póster.

Participantes

Podrán participar en este I Mini-Simposio becarios predoctorales y postdoctorales de investigación de la Facultad de Ciencias Experimentales (FCCEE) de la UAL. Aquellos estudiantes de grado o licenciatura realizando proyectos fin de grado o de carrera, así como aquellos becarios de colaboración que lo deseen, podrán también participar en la reunión, indicándolo adecuadamente en el envío de su resumen. Asistentes a este Mini-Simposio podrán ser todos aquellos miembros de la comunidad universitaria interesados en conocer las investigaciones más recientes llevadas a cabo en el campus de la UAL en las disciplinas de Ciencias Experimentales.

Inscripción

La inscripción al Mini-Simposio es gratuita y se hará rellenando el breve formulario disponible en http://www.ual.es/isimpos.

Plazos y presentación de los trabajos

La organización aceptará resúmenes tanto en español como en inglés. Los resúmenes deberán enviarse en formato MS-Word compatible con PC empleando la plantilla proporcionada en la Web y al final de este documento, de acuerdo con las instrucciones que figuran en ella. Los resúmenes están limitados a una única página. Al menos uno de los autores deberá estar inscrito como participante en el Mini-Simposio.

El resumen debe enviarse como fichero adjunto por correo electrónico a la a la dirección isimpos@ual.es, indicando en el asunto I MINI-SIMPOSIO DE INVESTIGACIÓN EN CIENCIAS EXPERIMENTALES, antes del 11 de Octubre de 2011. Esta dirección de correo electrónico está protegida contra los robots de spam, y se necesitará tener Javascript activado para poder verla.

El correo electrónico deberá especificar los siguientes datos:

- Nombre del autor que presenta la comunicación
- Título y
- Situación laboral (doctorado, posdoctorado, becario de colaboración, etc.)

El fichero adjunto debe ir identificado del siguiente modo: “Mini-Simposio-Apellido remitente"
El plazo de inscripción termina el 30 de octubre de 2011.

Los posters podrán ser entregados en la Facultad de Ciencias Experimentales desde el momento de formalizar la inscripción hasta el 4 de Noviembre de 2011.

Para ver el programa científico en detalle y más información visite la página web http://www.ual.es/isimpos. A continuación se resumen las fechas límite:

Fin de Inscripción	30 de Octubre de 2010
Fin de envío	18 de Octubre de 2011
Fin de entrega de posters	4 de Noviembre de 2011

Requisitos de los trabajos

Los resúmenes deberán enviarse en formato MS-Word compatible con PC empleando la plantilla proporcionada en la Web y al final de este documento, de acuerdo con las instrucciones que figuran en ella. Con los resúmenes de los trabajos se editara un libro de abstracts en el que aparecerán todos los resúmenes de los participantes. Todos los resúmenes serán presentados en forma de póster. La dimensión del mismo no será superior a 0.90 m de ancho y 1.20 m de alto. Los trabajos podrán venir firmados por varios autores, de los cuales uno de ellos será el contacto con la organización, que además será el encargado de defender su trabajo de investigación y de estar presente en la sesión programada al efecto.

Los posters serán impresos por los propios participantes y entregados en la Facultad de Ciencias Experimentales con fecha anterior al 4 de Noviembre de 2011. La organización se encargará de su exposición al público durante la semana de San Alberto en el Hall del Aulario IV desde el 8 de noviembre hasta el 15 de noviembre ambos inclusive.

Programa Científico

La reunión tendrá lugar el día de San Alberto Magno el 15 de Noviembre de 2011 (jornada de mañana). Para más información acerca del programa por favor consulte la página web: http://www.ual.es/isimpos.

El Comité Científico ser el encargado de valorar todos los trabajos presentados y seleccionar los 5 mejores para su posterior exposición oral. La exposición consistirá en un breve resumen de la investigación (temática, relevancia, equipo investigador, resultados más relevantes, etc.) no superior a 5 minutos (se pondrá a disposición de los seleccionados material informático y sala de grados para la presentación). El programa científico del Mini-simposio incluirá por tanto:

- Sesión de posters
- Sesión de exposiciones orales
- Sesión de premios

Premio

De todos aquellos pósteres enviados se seleccionarán los 5 mejores, atendiendo a los criterios de evaluación expuestos en las bases. La organización se encargará de recoger todos los pósters y de su exposición al público durante la semana de San Alberto en la Facultad de Ciencias Experimentales. Asimismo, se reserva el derecho a posteriores muestras, siempre con el propósito de promover la ciencia y divulgar el trabajo de los jóvenes investigadores.

Los premios se otorgarán por titulación (Ciencias Ambientales, Ingeniero Químico, Ingeniero de Materiales, Matemáticas y Químicas) por lo que habrá un premio de 300 euros para el póster ganador del mini-simposio en cada una de las titulaciones. La resolución de los mismos se dará a conocer el día de San Alberto en el acto de clausura, el 15 de noviembre de 2011. Los premiados deberán asistir obligatoriamente al acto de entrega de premios, pues se les pedirá resuman su trabajo en un tiempo aproximado de 5 minutos. La resolución de los premios se dará a conocer el mismo día.

Criterios de valoración y Jurado

Los trabajos serán valorados en función de tres criterios, carácter divulgativo, contenido y calidad científica así como presentación del mismo. El jurado estará compuesto por expertos investigadores de la Facultad de Ciencias Experimentales. La persona que defiende el poster deberá estar presente durante toda la sesión de presentación de posters, para poder optar así a los premios otorgados por la organización.

Aceptación de las bases

El hecho de concurrir a este concurso presupone la aceptación total de las presentes bases y la conformidad con las decisiones del jurado. La Facultad de Ciencias Experimentales podrá solicitar a los premiados la exposición de sus trabajos en futuras exposiciones, ferias o sesiones de divulgación de la Ciencia.

5. Programa

9:30h Entrega de documentación
Lugar: Hall de Aulario IV

10:00h Sesión de posters distribuida por secciones:
- Ingeniero de Materiales
- Ingeniero Químico
- CC Ambientales
- Matemáticas
- CC Química

Lugar: Hall de Aulario IV

12:00h Conferencia impartida por el Prof. Sixto Malato Rodríguez:
"Descontaminación de Agua mediante Energía Solar. Desarrollo, Aplicaciones y Expectativas"
Lugar: Sala de Grados, Aulario IV

13:00h Sesión de Exposiciones Orales
Lugar: Sala de Grados, Aulario IV

13:30h Entrega de premios y clausura
Lugar: Sala de Grados, Aulario IV

14:00h Copa de vino (cortesía de DICSA)
Lugar: Hall de Aulario IV

6. Lista de títulos de pósteres

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P02. How to fabricate a natural dye solar cell? A successful combination of materials — A.I. Maldonado Valdivia et al.

P03. New hybrid nanoparticles with optical and magnetic responses for surface enhanced raman spectoscopy (SERS) — B. Sierra-Martín et al.

Ingeniero Químico

P04. Influence of turbulence on the adaptation of the baculovirus–producer *Spodoptera Exigua* Se301 cell line to suspension culture — A. Beas-Catena et al.

P05. Continuous light profiles in tubular photobioreactor design — C. Brindley et al.

P06. Optimisation of culture conditions and medium for microalgae used in aquaculture — J. Camacho Rodríguez et al.

P07. Estudio de la degradación de paracetamol mediante foto-fenton a pH natural — I. Carra Ruiz et al.

P08. Aprovechamiento de gas de combustión para la producción de microalgas — R.M. Escudero Santiago et al.

P09. Bioactives from dinoflagellates microalgae — J.J. Gallardo Rodríguez et al.

P10. Carbon dioxide removal from combustion gases by gas-liquid absorption — C.V. González-López et al.

P11. Purification of structured triacylglycerols by vacuum short path distillation — E. Hita et al.

P12. Using the microalga *Scenedesmus almeriensis* in feeds for aquaculture: effect on growth and fatty acid composition of tissues in sea bream (Sparus aurata) — J.A. Jiménez et al.

P13. Enzymatic production of human milk fat substitutes — M.J. Jiménez et al.

P14. Isolation, morphological characterization and selection of heterotrophic microalgae for lipid production with industrial interest — N. Jiménez et al.

P15. Optimización del medio de cultivo de dinoflagelados mediante algoritmos genéticos — L. López Rosales et al.

P16. Production of oil as feedstock for biodiesel production from heterotrophic fed-batch fermentation of *Chlorella protothecoides* using glycerol as carbon source — M.D. Macías-Sánchez et al.

P17. B-cell hybridoma coculture within EL-4 thymoma cells enhances hybridoma cell growth and monoclonal antibody production — A. Martín-López et al.

P18. Effects of CD40 activation in B-cell hybridomas engineered to overexpress CD40: clonal variation for proliferation and monoclonal antibody production — A. Martín-López et al.

P19. Concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) by selective alcoholysis catalized by lipases — L. Martín et al.

P20. Captación de CO_2 mediante cultivo de microalgas a gran escala en reactores raceway — J.L. Mendoza et al.

P21. Efecto de la temperatura en la desinfección de aguas residuales por foto-fenton — E. Ortega et al.

P22. Development of a process for the production of l-aminoacids concentrates from microalgae by enzymatic hydrolysis — J.M. Romero García et al.

Licenciado en Ciencias Ambientales

P23. Regional controls of Ecosystem Functional Types richness in temperate South America — D. Alcaraz-Segura et al.

P24. The role of vegetation and lithology in the spatial and inter-annual response of EVI to climate in drylands of southeastern Spain — D. Alcaraz-Segura et al.

P25. The role of biological soil crusts on runoff, erosion and fertility in drylands — S. Chamizo et al.

P26. Biodiversidad en sistemas acuáticos artificiales: el caso de las balsas — I. Gallego et al.

P27. Effectiveness of amendments in remediation of a metal-arsenic polluted soil using *Lactuca sativa* l. Bioassays — V. González et al.

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P29. Sideróforos en tés de compost — F. Marín et al.

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P31. Respuesta hidrológica y erosiva a largo plazo de una cuenca de cárcavas. Predicciones ante el cambio climático — E. Rodriguez Caballero et al.

P32. Cambios espacio-temporales en el estado ecológico de los ríos Aguas y Almanzora: contraste de índices bióticos basados en macroinvertebrados — J. Rodríguez et al.

P33. An hydro-speleological approach to the study of mining impact on the Gypsum Karst of Sorbas (SE Spain) — L. Sanna et al.

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P34. Linear maps strongly preserving moore-penrose invertibility — M. Burgos et al.

P35. Aceleración del método del gradiente biconjugado para matrices dispersas en gpus — G. Ortega et al.

P36. Corneal topography reconstruction by generalized radial basis functions — D. Ramos López et al.

Licenciado en Químicas

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P38. Application of UHPLC-ORBITRAP-MS for the development of a fast screening analysis of veterinary drug residues in milk and powdered milk-based formulae — M.M. Aguilera-Luiz et al.

P39. Simultaneous determination of phytohormones in vegetables by ultra high performance liquid chromatography coupled to tandem mass spectrometry with polarity switching — M.I. Alarcón Flores et al.

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P42. Determination of chlorothalonil in difficult-to-analyse vegetable matrices using various multiresidue methods — N. Belmonte Valles et al.

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P44. $^{7}Li,^{15}N\{^{1}H\}$ HMQC NMR: Connecting nuclei at natural abundance! — M. Casimiro et al.

P01. EL AGUA SUBTERRÁNEA EN LA INGENIERÍA DE TÚNELES

E. M. López-Bautista, A. Pulido-Bosch

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Este trabajo fin de máster analiza una serie de aspectos relacionados con la construcción de túneles en presencia de agua subterránea, a través de la experiencia adquirida, con ejemplos concretos y cercanos. Los túneles analizados se ubican, mayoritariamente, en sistemas kársticos, dado su carácter hidrogeológico singular y su extensión en las zonas semiáridas de nuestro país. A partir de este análisis se concluye en la necesidad de predecir, con carácter previo al inicio de la excavación, la situación del nivel freático esperable, los posibles caudales de infiltración, los impactos probables en acuíferos, manantiales y corrientes de agua superficial, los problemas de la irrupción del agua en la excavación, y las presiones de infiltración (en caso de diseñarse un revestimiento permeable) o hidrostáticas (en caso de diseñarse estanco) que deberá soportar el túnel. Para ello se establece la necesidad del conocimiento y modelización hidrogeológica de la zona, que permita aunar esfuerzos y agudizar el ingenio para tomar decisiones constructivas adecuadas, en el momento oportuno, evitando daños medioambientales, mediante la adopción de las medidas correctoras necesarias, que permita concluir la obra con el mínimo impacto medioambiental y socioeconómico posible.

Agradecimientos. Al Catedrático D. Antonio Pulido Bosch por las enseñanzas recibidas durante el curso académico del Máster AQUARID 2010-2011, impartido por la Universidad de Almería, así como por la atención prestada durante la tutela del presente trabajo fin de máster y por sus comentarios y críticas efectuadas al manuscrito inicial. Al conjunto del profesorado del Máster AQUARID del curso 2010-2011, por las enseñanzas recibidas, con mención especial, a los coordinadores del mismo Prof. Dr. Francisco Sánchez Martos y Prof. Dr. Juan Gisbert Gallego por su dedicación. A mi jefe D. Elías Jiménez Burgos, de la empresa encargada de la conservación integral de las carreteras estatales de la provincia de Almería del sector AL-1, por su contribución y facilidades para la realización del máster. A todos aquellos anónimos trabajadores habituales de las obras subterráneas, por su esfuerzo, su dedicación y su contribución al avance de la técnica, a los que, frecuentemente olvidamos deseosos de ver la luz al final del túnel.

P02. HOW TO FABRICATE A NATURAL DYE SOLAR CELL? A SUCCESSFUL COMBINATION OF MATERIALS

A. I. Maldonado Valdivia, E. González Galindo, M. J. Ariza Camacho and M. J. García Salinas

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Dye-sensitized solar cells (DSCs) have been extensively studied because of their high energy conversion efficiency, simple fabrication process and low production cost[1]. DSCs are sandwich-type electrochemical cells, essentially composed by three elements: i) the working photoelectrode: a dye-sensitized mesoporous TiO_2 film deposited on a transparent fluorine-doped tin oxide (FTO) conducting glass; ii) an electrolyte solution containing iodide/triiodide ions as redox couple and iii) a platinized fluorine-doped tin oxide conducting glass (as counter electrode). The mesoporous TiO_2 film provides a large surface area for the adsorption of the dye molecules, and also allows the electrical connection with the redox electrolyte. Morphology of these mesoporous films, strongly affects conversion efficiency in DSCs, so their fabrication method is crucial to obtain highly efficient cells[2,3]. The most efficient dyes are based on bipyridyl complexes of transition metals, particularly ruthenium. However, natural dyes[4] extracted from fruits, vegetables or flowers can be an alternative low cost option.

In this work, the fabrication of a TiO_2 electrode is presented, using a natural dye extracted from bougainvillea bracts as sensitizer and Triton X-100 as thickener. Scanning Electron Microscopy (SEM) (Figure 1) and image-processing software were employed in order to investigate the morphology of the TiO_2 film. Film features were correlated with the photovoltaic performance of DSCs fabricated with this methodology. The electrical behavior of the performed solar cells were studied (Figure 2), obtaining efficiencies similar to previously reported data by others researchers[5].

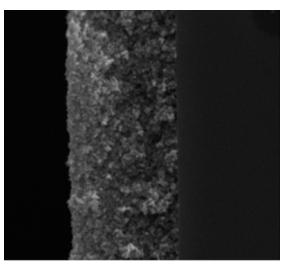

Figure 1: SEM images (10000X) of the cross-section of the sample.

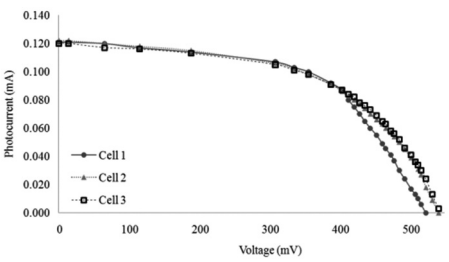

Figure 2: Photocurrent-voltage curves of three DSCs, employing a natural dye as sensitizer.

Acknowledgements. This research was supported by the Applied Physics Department of the University of Almería (Spain).

[1] Grätzel, Michael. *Dye-sensitized solar cells*. Journal of Photochemistry and Photobiology 4, 145-153, **2003**.

[2] Ito, Seigo; Kitamura, Takayuki; Wada, Yuji; Yanagida, Shozo. *Facile fabrication of mesoporous TiO_2 electrodes for dye solar cells: chemical modification and repetitive coating.* Solar Energy Materials & Solar Cells 76, 3-13, **2003**.

[3] Ito, Seigo; Nazeeruddin, M.K.; Zakeeruddin, S.M.; Péchy, Peter; Comte, Pascal; Grätzel, Michael; Mizuno, Takaki; Tanaka, Atsushi; Koyanagi, Tsuguo. *Study of Dye-Sensitized Solar Cells by Scanning Electron Micrograph Observation and Thickness Optimization of Porous TiO_2 Electrodes*. Int. J. of Photoenergy, Article ID 517609, **2009**.

[4] Furukawa, Shoji; Iino, Hiroshi; Iwamoto, Tomohisa; Kukita, Koudai; Yamauchi, Shoji. *Characteristics of dye- sensitized solar cells using natural dye*. Thin Solid Films, 518, 526-529, **2009**.

[5] Chang, Ho and Lo, Yu-Jen. *Pomegranate leaves and mulberry fruit as natural sensitizers for dye- sensitized solar cells*. Solar Energy, 84, 1833-1837, **2010**.

P03. NEW HYBRID NANOPARTICLES WITH OPTICAL AND MAGNETIC RESPONSES FOR SURFACE ENHANCED RAMAN SPECTOSCOPY (SERS)

B. Sierra-Martín, R. Contreras-Cáceres and A. Fernández-Barbero

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The optical and spectroscopic properties of noble metal nanoparticles stem from localized surface plasmons. This phenomenon occurs when electromagnetic field interacts with the electrons conduction band inducing a coherent oscillation of them. Two important consequences derivates of this delocalization; i) a strong absorption band appears in some region of the electromagnetic spectrum, and ii) an enhancement of the electromagnetic field near the nanoparticles surface. This is used in Surface Enhanced Raman Spectroscopy (SERS) to increase the Raman signal over 6 orders of magnitude in systems without hot spots and 10 orders of magnitude where hot spots are generated within the nanostructure. Herein, we present the application of a recently developed core@shell colloidal material comprising gold nanoparticles coated with a thermally responsive poly-(N-isopropylacrylamide) (pNIPAM) microgel, which are denote Au@pNIPAM.[1] While the gold cores provide the necessary enhancing properties, the pNIPAM shells can swell or collapse as a function of temperature, this change is expected to serve as a means of trap molecules and get them sufficiently close to the metal core for providing the SERS signal. This demonstration includes the firs report of the SERS spectrum of 1-naphthol, which had remained elusive to SERS ultra-sensitive analysis until now.1 It is important to note that the protective pNIPAM shell prevents electromagnetic coupling between metal particles, thereby restricting the detection limits. We have overcome this limitation with the fabrication of a SERS substrate comprising magnetic and silver particles encapsulated within a pNIPAM. This colloidal substrate has the ability to adsorb analytes from solution while it is expanded (low temperature) and reversibly generate hot spots upon collapse (high temperature or drying). Additionally, the magnetic functionality permits concentration of the composite particles into small spatial regions, which can be exploited to decrease the amount of material per analysis while improving its SERS detection limit. Proof of concept for the sequestration of uncommon molecular systems is demonstrated through the firs SERS analysis of pentachlorophenol (PCP), a chlorinated ubiquitous environmental pollutant.

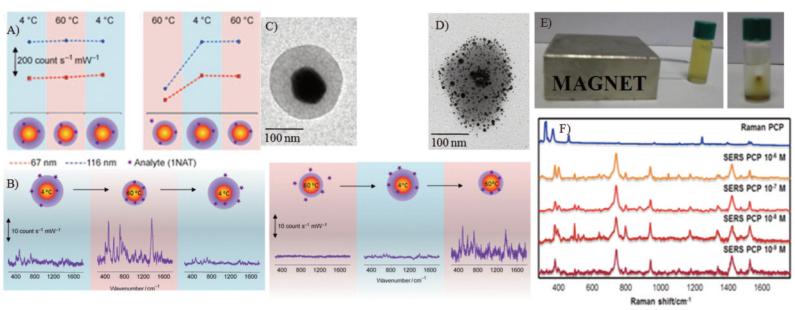

Figure 1. A) Variation of the intensity of the band at 1368 cm^{-1} of 1NAT as a function of gold core size for two temperature cycles; 4-60-4ºC (left), 60-4-60ºC (right). B) Variation of the SERS intensity of 1-naphtol for two temperature cycles; 4-60-4ºC (left), 60-4-60ºC (right). C) and D) TEM images of the Au@pNIPAM and Fe_3O_4@Ag@pNIPAM systems respectively. E) Optical image of the Fe_3O_4@Ag@pNIPAM before and after exposure to a permanent magnet. F) SERS ultradetection of PCP in dispersion of Fe_3O_4@Ag@pNIPAM after concentration using a permanent magnet.

Acknowledgments. Junta de Andalucía (project FQM-02353), Spanish Ministerio de Ciencia e Innovación MAT 2008-05755 and MAT 2009-14234-C03-02

[1] R. Contreras-Cáceres, B. Sierra-Martín, A. Fernández-Barbero, *Microsensors: Surface-Enhanced Raman Scattering Sensors base don Hybrid Nanoparticles*, In Tech, Rijeka (Croatia), **2011**.

P04. INFLUENCE OF TURBULENCE ON THE ADAPTATION OF THE BACULOVIRUS–PRODUCER *SPODOPTERA EXIGUA* Se301 CELL LINE TO SUSPENSION CULTURE

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One of the main pests affecting global crops is insects from the order Lepidoptera. In the greenhouses of Almeria one of the most important pest is *Spodoptera exigua*, which feeds mainly on peppers and watermelons, causing important damage to crops and economic losses. The way to deal with plagues have been through the use of chemical pesticides of broad–spectrum; however, as they are being banned due to safety reasons, they must be replaced with other means of control safer to people and to the environment. One of the means proposed is the use of baculoviruses, which are pathogenic only to Lepidoptera and most frequently specific only against one species. This is the case of the nucleopolyhedrovirus of *Spodoptera exigua* (SeMNPV), pathogenic to larvae of *Spodoptera exigua*. Nowadays, SeMNPV is commercially produced exclusively *in vivo*, by the company Biocolor S.L. A promising alternative is the *in vitro* production of the baculovirus. However, a cheap mass production process needs the use of suspended culture in classic bioreactors.

Shaking at Re number in the range 3,500–7,500, we have successfully adapted static cultures of the *Spodoptera exigua* Se301 cell line to suspension. This cell line has proven to be very sensitive to shear stress and thus the use of protective reagents was necessary. The additives which offered the highest level of protection were polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP). When adding PVP or PVA to shaken cultures maximal cell densities and apparent growth rate (μ_{app}) showed no apparent differences when compared to static cultures. A typical effect of agitation is clumping. Se301 cells clump severely when agitated, therefore the use of anti-clumping agents was required, being the most efficient dextran sulfate (DS).

Se301 cells have been infected in static and suspended cultures, cultured in both serum-free and serum-containing media with SeMNPV at low multiplicities of infection (MOIs), obtaining a two-fold increase on occluded virus (OB) production in shaken cultures.

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P05. CONTINUOUS LIGHT PROFILES IN TUBULAR PHOTOBIOREACTOR DESIGN

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Designing efficient photobioreactors (PBRs) is critical for the cost-effective production of microalgae. The growth of photosynthetic cells inside PBRs is a complex system combining the effects of photosynthesis, fluid-dynamics and irradiance distribution within the PBR. In dense cultures, the light intensity decreases exponentially from the illuminated surface to the centre of the reactor because of the mutual shading effects of cells. Closed PBRs need intense mixing conditions in order to favour the exposure of cells to light by introducing fluctuations in light; knowing how photosynthesis rate responds to these fluctuations is essential for the evaluation of PBR performance. A recent work has studied continuous light distributions, showing that light regimes that are equivalent in frequency, illuminated PBR fraction, incident irradiance and light availability can produce different photosynthetic responses, depending on the shape of the irradiance profile that the cells "see".[1] The present work combines fluid-dynamic and light regime principles to produce a tool for the design and analysis of tubular PBRs.

The culture systems used were two outdoor horizontal tubular PBRs: one of 220 L working volume and 5 cm of internal tube diameter, and the other one of 50 L and 2.5 cm.[2] The photosynthetic response data considered was reported by Brindley et al.[3] The mechanistic model proposed by Camacho et al.[4] was also used to evaluate photosynthetic responses to different light patterns, the general equation admitting continuous light profiles as well as intermittent light profiles. Two types of light profiles were evaluated: a square-wave irradiance profile (discontinuous) and a continuous function that simulates the actual irradiance profile that cells experiment in dense cultures in tubular PBRs.[1] Hypothetical conditions were considered for the evaluation of the fractional productivity (P/P_{max}, where P is predicted for a given light regime, species and PBR, and P_{max} is a model-parameter). The general equation of the model can be expressed in terms of *I(t)*, the irradiance-time profile of an average cell in a tubular PBR, and the fractional productivity was solved by Simpson integration. The predictions present over 30% difference, even though both light patterns are equivalent in terms of cycle duration, incident irradiance, and illuminated fraction, only differing in the shape of *I(t)*. Henceforth, predictions will be made using the actual profile.

Fluid-dynamics and light regime principles were combined to produce a design tool for the analysis of tubular PBR performance, following these steps: calculation of the irradiance profile in the PBR (irradiance distribution model); calculation of ϕ; estimation of the frequency (fluid-dynamic principles); combination of the actual irradiance profile and the light regime variables to obtain I(t); prediction of the photosynthetic response. This procedure was validated by checking the correlation between the predicted and the observed data. Finally, the design tool was used to analyze the effect of the tube radius on areal productivity. The results show that the size of the PBR has a limited influence on the maximum areal productivity.

Acknowledgements. This research work was funded by Ministerio de Ciencia e Innovación, grant CTQ2008-06741-CO2.

[1] C. Brindley, F. G. Acién Fernández and J. M. Fernández-Sevilla, *Bioresour. Technol*. **2011**, 102, 3138-3148.

[2] F. G. Acién Fernández, F. García Camacho, J. A. Sánchez Pérez, J. M. Fernández Sevilla and E. Molina Grima, *Biotechnol. Bioeng*. **1998**, 58, 605-616.

[3] C. Brindley, F. G. Acién Fernández and J. M. Fernández-Sevilla, *Biotechnol. Bioeng*. **2010**, 106, 228-237.

[4] F. Camacho Rubio, F. García Camacho, J. M. Fernández Sevilla, Y. Chisti and E. Molina Grima, A mechanistic model of photosynthesis in microalgae, Biotechnol. Bioeng. **2003**, 81, 459-473.

P06. OPTIMISATION OF CULTURE CONDITIONS AND MEDIUM FOR MICROALGAE USED IN AQUACULTURE

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Nannochloropsis gaditana and *Isochrysis galbana* are microalgae with a high nutritional value and a content of pigments and PUFAs[1] that make them interesting for aquaculture. In order to maximise their productivity and nutritional value in large scale culture, a medium optimisation has been carried out by adapting a commercial medium (Algal) to the most favourable nutrient level. For this, we carried out experiments in 2L bubble column photobioreactors operated indoors in continuous mode (0.3 d^{-1}) and illuminated simulating the solar cycle with a maximum irradiance 1000 $\mu Em^{-2}s^{-1}$ at 25 ºC. The nutrient levels have been specifically adapted to each microalga by modifying the nitrogen content from 2 mM to 16 mM (NO_3^-), maintaining the proportion in other nutrients.

The optical parameters were measured: K_a[2] (light attenuation coefficient) and I_{av} (average irradiance), being both inversely proportional to the biomass concentration, obtaining K_a values from 0.158 to 0.177 $m^2 \bullet g^{-1}$ in *N. gaditana* and from 0.162 to 0.169 in *I. galbana*. I_{av} was in a range from 81 to 155 $\mu E \bullet s^{-1} \bullet m^{-2}$ in *N. gaditana* and from 149 to 230 $\mu E \bullet s^{-1} \bullet m^{-2}$ in *I. galbana*. Once the biomass was harvested, lipid conteng, proteins, anshes and carbohydrates were measured.

The optimal growth conditions for *N. gaditana* were observed for $[NO_3^-]$ = 11.3 mM while for *I. galbana* the optimum was $[NO_3^-]$ = 4 mM. The maximum biomass productivities were 0.7 $gL^{-1}d^{-1}$ for *N. gaditana* and 0.35 $gL^{-1}d^{-1}$ for *I. galbana*. The most favourable fatty acid profile for both strains was also found under these growth conditions, obtaining around 11.3 % (d.w.) and 13.2 % (d.w.) of total fatty acids in *N. gaditana* and *I. galbana* biomass respectively while the relative EPA content in optimal conditions in *N. gaditana* was 37 % and the relative DHA[3] content in optimal conditions in I. galbana was 9.5 %.

A high biomass productivity and a balanced lipid content for application to aquaculture of the selected strains has been achieved, but the protein level measured is still not sufficient. Therefore new culture conditions should be tested (as irradiance, temperature and dilution rate) to obtain an optimal biomass for aquaculture.

Acknowledgments. This work has been supported by the Consejería de Innovación, Ciencia y Empresa of the Junta de Andalucía (P09-AGR-5334), the European Regional Development Fund Programme.

[1] Jorge M. S. Rocha, Juan E. C. García, Marta H. F. Henriques, in "*Growth aspects of the marine microalga Nannochloropsis gaditana*", Biomolecular Engineering, Vol. 20, **2003**; pp. 237–242.

[2] Molina E., Fernández J.M., Sánchez J.A., García F., in *"A Study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances"*, J. Biotechnol, Vol. 1, **1996**; pp. 59–69

[3] Vishwanath Patil, Torsten Källqvist, Elisabeth Olsen, Gjermund Vogt, Hans R. Gislerod, in *"Fatty acid composition of 12 microalgae for possible use in aquaculture feed"*, Aquacult Int, Vol. 15, **2007**; pp. 1-9.

P07. ESTUDIO DE LA DEGRADACIÓN DE PARACETAMOL MEDIANTE FOTO-FENTON A pH NATURAL

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Los Procesos de Oxidación Avanzada (PPOA) han demostrado ser efectivos en la degradación de contaminantes orgánicos, siendo especialmente interesantes los PPOA capaces de utilizar el Sol como fuente de energía. De entre dichos procesos, destaca el proceso de fotocatálisis homogénea, foto-Fenton. Se trata de un proceso en el que el Fe (II), que actúa como catalizador, reacciona con H_2O_2, generando radicales hidroxilo ($HO^{\cdot}$) que oxidan los contaminantes hasta CO_2 y H_2O, todo ello favorecido por la luz (ecuaciones 1 y 2). Sin embargo, el pH óptimo de operación es 2.8, lo que supone mayores costes de operación, junto con el impacto ambiental resultante. Por ello, muchos autores están estudiando sistemas de foto-Fenton modificado en el que se utilizan quelantes del hierro estables a pH más elevados[1].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{-} + HO^{\cdot} \qquad (1)$$
$$Fe^{3+} + H_2O + h\upsilon \rightarrow Fe^{2+} + H^{+} + HO^{\cdot} \qquad (2)$$

En este trabajo se estudia la oxidación de paracetamol mediante un proceso de foto-Fenton a pH natural en planta piloto, ya que es un contaminante que no se ve afectado por tratamientos de aguas convencionales. Se evalúa su degradación utilizando como fuente de hierro sulfato ferroso y se compara el resultado obtenido utilizando otros compuestos de hierro evaluados previamente a escala de laboratorio: d-gluconato de hierro, lactato de hierro, mesilato de deferoxamina y Ferroactiv (fertilizante). Se ha estudiado también el modo de adición del peróxido de hidrógeno (una adición inicial, dos adiciones, adición continua) en planta piloto, ya que se ha visto que la forma de adición influye significativamente en la mineralización cuando se trabaja a pH 2.8[2].

Mediante experimentos en simulador solar para estudiar las distintas fuentes de hierro, se descartaron el fertilizante y el mesilato de deferoxamina por la coloración y el alto carbono orgánico disuelto (COD) que aportan y se procedió a realizar los experimentos en planta piloto exterior. En la figura 1 se muestran las mineralizaciones obtenidas en planta piloto. Se puede ver que para los experimentos con sulfato ferroso el pH inicial y la forma de adición de peróxido de hidrógeno no afectan al resultado final, mientras que el paso a condiciones externas afecta a la mineralización cuando se utilizan el gluconato y lactato de hierro. En estos dos últimos casos, la relación peróxido de hidrógeno y COD iniciales no resultó ser óptima para estos sistemas.

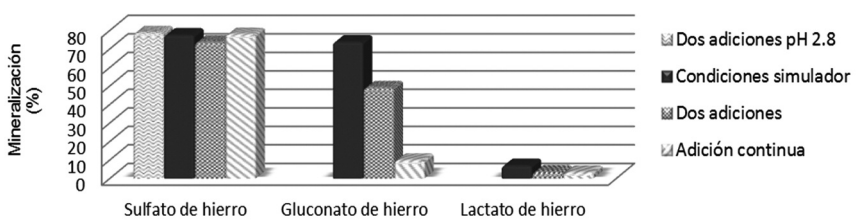

Figura 1. Comparación entre las mineralizaciones obtenidas en los experimentos en planta piloto.

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[1] J.J. Pignatello, E. Oliveros, A. MacKay, *Environ. Sci. Technol.* **2006**, *36*, 1-84.

[2] L. Santos-Juanes, J.L. García Sánchez, J.L. Casas López, I. Oller, S. Malato, J.A. Sánchez Pérez, *App. Cat. B: Environ.* **2011**, *104*, 316–323.

P08. APROVECHAMIENTO DE GAS DE COMBUSTIÓN PARA LA PRODUCCIÓN DE MICROALGAS

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El CO_2 atmosférico ha ido aumentando paulatinamente como consecuencia de la actividad industrial y de la gran dependencia del petróleo de la sociedad actual.

Las microalgas, necesitan CO_2 para su crecimiento, por lo que se plantea la producción de microalgas como una oportunidad para reducir el contenido de CO_2 atmosférico en entornos industriales. Por otro lado las microalgas son fuente de lípidos y proteínas, que pueden rentabilizar aún más el proceso.

Para llevar a cabo esta investigación, se están utilizando reactores raceway con capacidad para 1200 y 4400 litros. El primer reactor, el de menor volumen, donde se alcanzan mayores productividades, debido a que la exposición celular a la luz es mayor, además los distintos cambios de nivel que presenta el reactor a lo largo de su recorrido aumentan la turbulencia del cultivo, favoreciendo así la desorción de oxígeno. El segundo raceway es un raceway horizontal, de mayor volumen, el cultivo consta de un espesor de 10 cm en la mayor parte de su recorrido, esto hace que las células estén en menor contacto con la luz solar. Ambos reactores cuentan con un foso de aproximadamente 1000 litros, en el que se inyecta aire o CO_2 dependiendo de la demanda y de las condiciones del cultivo. La finalidad de este foso, entre otras es la mejora de la transferencia de materia entre la fase líquida y la fase gaseosa.

Tras la caracterización de ambos reactores y la mejora de los burbujeadores de aire/CO_2 se llevo a cabo el seguimiento del cultivo utilizando CO_2 puro durante varias semanas, para ensayar a distintas velocidades de dilución. Una vez comprobado el correcto funcionamiento y alcanzado varios estados estacionarios, se ha comenzado a usar CO_2 de combustión proveniente de una instalación que permite generar gases de combustión a partir de la quema de gasoil utilizando una caldera semi-industrial. Los gases de combustión se enfrían y se comprimen almacenadlos a 2 bares en un depósito. Estos gases de combustión están exentos de SO_x, bajo contenido de NO_x y presenta un 8% de CO_2.

Una vez realizados varios ensayos con CO_2 de combustión alcanzando distintos estados estacionarios estables que puedan ser comparativos con los obtenidos con CO_2 puro, se procede a analizar los posibles efectos tóxicos acumulativos que se han podido generar en los cultivos por la utilización de CO_2 de combustión.

Tabla 1. Resumen de pesos secos tras una semana de experimentación con CO2 de combustión.

	RWinclinado			RWhorizontal		
CO_2	C(g/l)	Productividad (g/día·m^2)	Productividad (kg/año·m^2)	C(g/l)	Productividad (g/día·m^2)	Productividad (kg/año·m^2)
Puro	3.4	30.91	11.12	0.97	19.4	6.98
Combustión	1.9	17.27	6.21	1.07	21.52	7.74

Las productividades anteriores corresponden a un ensayo en el que se diluye el 15% del reactor, se observa que en las condiciones más favorables de temperatura y luminosidad, la productividad en el reactor pequeño disminuye con CO_2 de combustión alrededor de un 43% y para el caso del reactor horizontal apenas se aprecian diferencias. Estos datos no son concluyentes.

Actualmente se sigue trabajando en esta línea, y se están obteniendo datos más favorables. No es posible facilitarlos en este momento ya que aun se siguen analizando y tratando los resultados obtenidos.

P09. BIOACTIVES FROM DINOFLAGELLATES MICROALGAE

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More than 21,000 structurally diverse natural products with a diverse array of bioactivities have been discovered from the sea in the last four decades. However, this extraordinary wealth could not be exploited due to several significant limitations that, in most of the cases, still remain. Dinoflagellate microalgae are known to be the most important source of marine biotoxins. Blooms of toxic dinoflagellates are associated with fish kills, poisonings of marine wildlife, and seafood poisonings of humans. Dinoflagellate-associated human poisonings include paralytic shellfish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, and ciguatera fish poisoning.

Bioactives from dinoflagellates are of increasing interest because of their high commercial value, their influence on safety of seafood, and their use in biomedical, toxicological and chemical research programs. The potential applications of dinoflagellate toxins described up to now are incredibly diverse. However, this potential has not been exploited for the time being and its real magnitude remains undiscovered. The lack of sufficient quantities of toxins for research purposes hinders the biological characterization of the new molecules described in the last years. Bioreactor culture of fragile and slow-growing dinoflagellates is pointed out as the most suitable method to produce these metabolites. A protocol for strain characterization, culture optimization and scale-up using *P. reticulatum* as dinoflagellate model is proposed.

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P10. CARBON DIOXIDE REMOVAL FROM COMBUSTION GASES BY GAS-LIQUID ABSORPTION

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The great industrial development in the last century has contributed to the increase of CO_2 emissions, which have raised 80% from 1970 to 2004. This gas is responsible for more than 70% of the enhanced greenhouse effect[1]. Therefore, industries are conducting research on technologies to reduce these emissions. Nowadays, chemical absorption with amine solvents is the most used technology. However, amines are toxic and corrosive and they require high amounts of energy to be regenerated[2]. There are also processes based on sodium/potassium carbonate, which have a lower heat of regeneration than amines but its rate of reaction is slower[3]. Biological technologies to remove CO_2 from flue gases are an alternative of increasing interest[4]. In this sense, a new process for CO_2 capture from flue gases that can be coupled with microalgae-based processes has been developed and patented[5].

This work presents a comparison of CO_2 absorption using different aqueous solutions in a small scale bubble column. It is possible to achieve CO_2 removal efficiencies higher than 70%. The use of carbonated solutions allows capturing CO_2 with high efficiency and they can also be regenerated by a biological treatment so it is advantageous. This system was scaled-up and compared with a packed tower, as this is one of the most used equipments at industrial scale for this kind of process. We checked that the presence of contaminants in flue gases such as NO, NO_2 or SO_2 at low concentrations did not affect the CO_2 absorption, as the buffering effect of the medium allows absorbing these gases without changing the pH of the liquid. We obtained similar CO_2 depuration efficiencies in packed tower and bubble column (Table 1).

Table 1. Comparison of CO_2 depuration in packed tower and bubble column.

System	*CO_2 depuration, %*	*L/G, l/l*	*TIC, g/l*
Packed tower	65	2.5	0.02
Bubble column	60	0.04	0.87

In packed tower we achieved 65% CO_2 depuration and a concentration of absorbed inorganic carbon in the liquid phase of 0.02 g/L for a liquid/gas ratio of 2.5. On the other hand, in bubble column we achieved a similar CO_2 depuration of 60% but the concentration of absorbed inorganic carbon was of 0.87 g/L and the liquid/gas ratio was of only 0.04. In bubble column the liquid residence time can be controlled by regulating the liquid flow rate, so it is possible to enhance the concentration of absorbed inorganic carbon in the liquid. However, the energy consumption in the gas impulsion is higher than the required in packed tower. Nonetheless, the use of a bubble column is advisable because the volume of liquid per volume of gas processed is 50 times lower than in packed tower so the dimensions of both capture and biological treatment systems would be smaller.

Acknowledgements. ENDESA S.A., CDTI, Ministerio de Educación y Ciencia (CTQ2004-07628-C02-01/PPQ), Junta de Andalucía and Plan Andaluz de Investigación (BIO 173).

[1] IPCC, in *"Climate Change 2007: Mitigation*, B. Metz, O.R. Davidson, P.R. Bosch, R. Dave and L.A. Meyer (Eds.), Cambridge University Press (UK), **2007**.

[2] M. R. M. Abu-Zahra, L. H. J. Schneiders, J. P. M. Niederer, P. H. M. Feron and G. F. Versteeg. *Int. J. Greenh. Gas Con*. **2007**, 1, 37-46.

[3] Y. Liang, D. P. Harrison, R. P. Gupta, D. A. Green and W. J. McMichael. *Energ. Fuels*. **2004**, 18, 569-575.

[4] C. V. González López, F. G. Acién Fernández, J. M. Fernández Sevilla, J. F. Sánchez Fernández, M. C. Cerón García and E. Molina Grima. Bioresource Technol. **2009**, 100, 5904-5910.

[5] F. G. Acién, E. Molina, J. M. Fernández-Sevilla, C. V. González, B. Llamas and J. C. Ballesteros. Liquid-phase gas collection. Patent WO/2009/112624, **2009**.

P11. PURIFICATION OF STRUCTURED TRIACYLGLYCEROLS BY VACUUM SHORT PATH DISTILLATION

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The ultimate goal of this work is to purify, by high vacuum short path distillation, structured triacylglycerols (STAGs) and free fatty acids (FFAs).

The interest in the production of the STAG for nutritional and therapeutic purposes is that the absorption of fatty acids not only depends on the type of acid, but also the position it occupies in the TAG molecule. In this work we used tuna oil because it is rich in docosohexaenoico acid (DHA), which is particularly important for nervous system development.

The separation of the reaction products is an important aspect, which is often afforded less importance. The major products of an acidolysis reaction are FFAs and TAGs. In previous work the separation of FFAs and TAGs has been tested by extraction of the former with water–ethanol mixtures. In this method the most important variable to optimize is the water/ethanol ratio, which depends on the type of FFAs and TAGs[1].

In this case, the separation of the product from acidolysis between caprylic acid and tuna oil catalyzed by lipase DF from *Rhizopus oryzae* immobilized on Accurel MP1000 was carried out in a bench-scale short path distiller (SPD). This distillation is a separation process that uses very high vacuum (up to 10-3 mbar), reducing the evaporation temperature and the possible adverse thermal effects on the products.

First, distillation diagrams of caprylic acid, tuna oil and binary mixtures caprylic acid-tuna oil were experimentally obtained. The most important variable is evaporation temperature, keeping constant the other variables (feeding flow rate about 1 L/h, evaporator pressure, 0.001 mbar, condensation temperature, 50 ºC, feeding temperature, 50 ºC and agitation rate, 300 rpm).

The diagram obtained indicates that, in these conditions, the separation of TAGs from the other lipids could be complete at evaporation temperatures between 180 and 240 ºC. Thus, in the separation of a mixture tuna oil-caprylic acid 1:1 w/w at 150 ºC were obtained 96.5% pure TAGs in the residue and 98.6% pure fatty acids in the distillate

All results obtained from the diagrams of distillation we have provided the basis to separate the product from the acidolysis between caprylic acid and tuna oil, obtained after two steps of the fraction rich in the triacylglyceride structured (STAG) at 170 °C for distiller, a TAGE with a purity of 87% and a global yield after two passes of 80%.

[1] Hita, E.,Robles, A., Camacho, B., González, P.A., Esteban, L., Jiménez, M.J., Muñío, M.M., Molina, E. *"Production of structured triacylglycerols by acidolysis catalyzed by lipases immobilized in a packed bed reactor"*, Biochemical Engineering Journal, Vol. 46, **2009**; pp. 257–264.

P12. USING THE MICROALGA *SCENEDESMUS ALMERIENSIS* IN FEEDS FOR AQUACULTURE: EFFECT ON GROWTH AND FATTY ACID COMPOSITION OF TISSUES IN SEA BREAM (*SPARUS AURATA*)

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A nutritional assay has been conducted during forty five days with sea bream juveniles using biomass of *Scenedesmus almeriensis*. The experimental diets were formulated to have 0% (control). 25% (SC25), 37% (SC37), 50% (SC50) and 75% (SC75) of fish meal protein replaced by algal biomass in order to analyze the effect on growth and body fatty acid composition. Zootechnical parameters were not affected by *S. almeriensis* inclusion. Growth of fish fed on SC37 was higher than those of SC25 and SC75, but without significant differences compared to control (Table 1). Fish fed on algal meal-based diet showed higher 18:2n6 and 18:3n3 and lower 20:5n3 and 22:6n3 levels in their muscle than control fish (Table 2). Results indicate that *S. almeriensis* can replace up to 37% fish meal protein in diets for sea bream juveniles with no negative effects on fish performance.

Table 1. Zootechnical parameters of fish fed the experimental diets.

	Control	SC25	SC37	SC50	SC75
P_{final} (g)	25.0 ± 3.8^{ab}	23.4 ± 3.6^{a}	26.6 ± 3.5^{b}	25.0 ± 2.9^{ab}	24.1 ± 4.4^{a}
SGR	1.02 ± 0.05^{ab}	0.91 ± 0.03^{a}	1.1 ± 0.06^{b}	1.02 ± 0.03^{ab}	0.98 ± 0.06^{ab}
ADG	0.18 ± 0.01^{ab}	0.16 ± 0.01^{a}	0.20 ± 0.01^{b}	0.18 ± 0.01^{ab}	0.10 ± 0.01^{a}
IC	1.92 ± 0.22^{ab}	2.11 ± 0.06^{b}	1.7 ± 0.03^{a}	1.86 ± 0.14^{ab}	1.91 ± 0.18^{ab}
PER	0.95 ± 0.1^{ab}	0.86 ± 0.03^{a}	1.07 ± 0.02^{b}	0.98 ± 0.07^{b}	0.96 ± 0.1^{ab}
WG	204.5 ± 7.0^{ab}	188.9 ± 3.7^{a}	216.3 ± 8.3^{b}	204.4 ± 4.6^{ab}	198.6 ± 7.81^{a}
IHS	2.3 ± 0.38^{b}	2.09 ± 0.44^{b}	2.23 ± 0.45^{b}	2.06 ± 0.36^{b}	1.82 ± 0.39^{a}
IVS	8.04 ± 0.92^{b}	7.31 ± 1.05^{ab}	7.99 ± 1.25^{b}	7.47 ± 1.01^{b}	7.32 ± 1.07^{a}

Table 2. Fatty acid profile of fish fed the experimental diets.

	S. almeriensis	Initial	Control	SC25	SC37	SC50	SC75
14:00	3.9 ± 0.1	3.8 ± 0.1^{d}	3.5 ± 0.1^{c}	3.3 ± 0.2^{c}	3.2 ± 0.1^{b}	3.1 ± 0.1^{b}	3.0 ± 0.2^{a}
16:00	19.7 ± 0.1	20.8 ± 0.1^{a}	22.3 ± 0.5^{b}	21.7 ± 0.3^{ab}	22.0 ± 0.5^{b}	21.7 ± 0.2^{ab}	22.2 ± 0.5^{b}
18:00	4.5 ± 0.2	6.2 ± 0.1^{c}	6.0 ± 0.2^{abc}	5.9 ± 0.2^{a}	6.0 ± 0.2^{abc}	6.0 ± 0.1^{a}	6.4 ± 0.3^{c}
18:1n9	10.4 ± 0.1	15.9 ± 0.1^{a}	23.0 ± 0.3^{b}	23.8 ± 1.0^{b}	23.9 ± 0.4^{b}	23.2 ± 0.6^{b}	24.3 ± 1.0^{b}
18:2n6	8.4 ± 0.1	6.5 ± 0.1^{a}	6.9 ± 0.2^{a}	7.3 ± 0.3^{b}	7.3 ± 0.1^{b}	7.5 ± 0.1^{b}	7.9 ± 0.4^{c}
18:3n3	5.0 ± 0.1	0.9 ± 0.1^{a}	1.0 ± 0.1^{a}	2.2 ± 0.5^{b}	2.6 ± 0.1^{c}	2.6 ± 0.1^{c}	3.2 ± 0.6^{d}
20:4n6	1.2 ± 0.1	1.6 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
20:5n3	8.8 ± 0.1	11.1 ± 0.1^{c}	7.6 ± 0.2^{b}	7.1 ± 0.3^{a}	6.9 ± 0.2^{a}	7.1 ± 0.2^{a}	6.9 ± 0.3^{a}
22:6n3	14.0 ± 0.2	20.0 ± 0.4^{c}	14.3 ± 0.4^{b}	13.9 ± 0.8^{ab}	13.7 ± 0.3^{a}	14.4 ± 0.6^{ab}	13.9 ± 0.8^{ab}

^{abc} Different letters on the same row indicate significant differences among treatments ($P < 0.05$).

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P13. ENZYMATIC PRODUCTION OF HUMAN MILK FAT SUBSTITUTES

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Lipids contained in human milk (98% triacylglycerols, TAGs) represent the main source of energy for the breastfed baby. Palmitic and oleic acids (PA and OA) are the two most abundant fatty acids (FAs); PA represents about 20-25% of the total milk FAs and is located primarily at the *sn*-2 position (>60%), whereas the *sn*-1,3 positions are mainly occupied by OA. Docosahexaenoic acid (DHA) is also present in human milk fat (HMF, 0.1-1.8%) and mainly esterified at the central position of TAGs (>66%). According to numerous studies, the distribution of PA at *sn*-2 position is of great importance for the absorption of fat and minerals in infants. In addition, a sufficient provision of DHA is thought to be essential for optimal visual and neurological development during early life.

The aim of this work was to obtain human milk fat substitutes (HMFS) rich in PA at *sn*-2 position and OA at *sn*-1,3 positions from palm stearin (PS) and rich in PA and DHA at *sn*-2 position and OA at *sn*-1,3 positions from tuna oil (TO).

The developed process consists of two enzymatic reactions. In the first one, acidolysis of PS or TO and PA catalyzed by Novozym 435 from *Candida antarctica* was studied. For this reaction, influence of temperature, hexane/reaction mixture ratio, reaction time and placement of lipase (dispersed or contained in a cartridge filter in a 1 L stirred tank reactor, STR) were tested. TAGs with 74% PA at *sn*-2 position were obtained using 1:1 PS/PA weight ratio, 10% (w/w) lipase/reaction mixture ratio and 10 mL hexane/g reaction mixture at 37 ºC for 48 h with the enzyme dispersed in the reaction medium. TAGs with 61-63% PA and about 10% DHA, mainly located at *sn*-2 position, were obtained using the same conditions from TO in both reactors types. Then, neutralization of free fatty acids (FFAs) by KOH hydroethanolic solutions provided 100% pure TAGs, which were used in the following step. This involved the displacement of PA located at *sn*-1,3 positions of TAGs by acidolysis with OA catalyzed by the *sn*-1,3 specific lipase DF from *Rhizopus oryzae* immobilized on Accurel MP1000. Influence of FFA/TAG molar ratio, purity of OA FFAs and intensity of treatment (IOT, lipase amount × reaction time / TAGs amount) were studied in this case. Under the best conditions (6:1 OA 90%/TAG molar ratio, 10 mL hexane/g TAG, 0.4 g lipase × h/g TAG and 37 ºC), STAGs with 67% OA at *sn*-1,3 positions and 66% PA at *sn*-2 position were obtained from TAGs with 74% PA equally distributed. In addition, STAGs with 52% PA and 15% DHA at *sn*-2 position and also 67% OA at *sn*-1,3 were obtained in both reactors types from TAGs derived from the initial acidolysis of TO. Finally, STAGs were purified again neutralizing the FFAs (100% purity, yields of over 80%).

With all this, STAGs with a similar composition and distribution of PA and OA to that of HMF were obtained. In addition, STAGs rich in DHA, mainly located at *sn*-2 position, were produced starting from TO.

P14. ISOLATION, MORPHOLOGICAL CHARACTERIZATION AND SELECTION OF HETEROTROPHIC MICROALGAE FOR LIPID PRODUCTION WITH INDUSTRIAL INTEREST

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Climate change is the greatest environmental threat of this century. One of the main causes of it is the fossil fuels whose combustion brings about a permanent deployment of toxic to our environment. As a solution to this, biofuels emerged. Microalgae are microorganisms that apart from generating potential biofuels, feed and other high-value substances are useful in bioremediation applications and as biofertilizers fixing nitrogen.

This work had as main objective isolate heterotrophic microalgae of samples of marine natural Park of Almeria and realize a characterization and identification of microalgae suitable for producing lipids with industrial interest. For this, it was isolated marine and freshwater microalgae, for obtaining fatty acid profile of all specimens and to know their biotechnological potential. Then culture conditions were optimized to improve biomass production and fatty acid profile of selected specimens. The specimens have been selected on the basis of a number of parameters such as a content of fatty acids obtained, content of biomass concentration in 7-10 days, convergence of rapeseed oil and olive and biotechnological interest of fatty acid profile and will be subjected to agitation, aeration in some cases and different concentrations of inorganic nutrients and organic carbon bases in order to increase the concentration of biomass and the percentage of fatty acids. The specimens are designated by acronyms (A1, AF1, CG1 y FLG), to not being identified by sequencing, so the specie is unknown and gender a priori. It has been studied the influence of five different culture media in heterotrophic growth as nutritional model, using as sources of carbon glucose and glycerol. To optimize the culture conditions for improving the biomass productivity and fatty acids content of isolated specimens.

The biomass concentration was determined by dry weight method and the fatty acid profile was obtained by gas chromatography using the method of direct methylation. The data were statistically analyzed using the software Statgraphics-plus 5.1. Data showed that with the specimen FLG a high biomass productivity of 0.76 g/L·d is achieved with agitation (75 rpm), double concentration of inorganic nutrients and glycerol (30g/L) and the total content of fatty acids achieved in dry weight (100 rpm) was 19% (high in PUFAs, 32% EPA and 27% DHA). Specimen A1 gives the highest values of total content of fatty acids in dry weight (9%) when is grown with glycerol (30g/L) and agitated (100 rpm), being 31% palmitic and 51% DHA. The growth of the specimen CG1 is favored with a value of high agitation (200rpm), double concentration of inorganic nutrients and glucose (30g/L). Getting a biomass concentration of 6 g/L, a total fatty acid content in dry weight of 31%, a convergence of rapeseed oil and olive high (81.4% and 71.2% respectively) and 50% of oleic acid. Finally, AF1 specimen is very interesting because total fatty acid content in dry weight of 41% is achieved in static condition, being the oleic acid the majority and convergences of rapeseed oil and olive oil very high (87.3% and 82.5% respectively).

The present work has as objective to contribute in the microbial diversity of our country to perform an isolation of heterotrophic microorganisms of a natural park, at the same time characterizing the microalgae obtained and finally making a lipid profile to a subsequent application in the different areas already mentioned.

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P15. OPTIMIZACIÓN DEL MEDIO DE CULTIVO DE DINOFLAGELADOS MEDIANTE ALGORITMOS GENÉTICOS

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Los dinoflagelados son microalgas que producen toxinas y bioactivos de gran interés para programas de seguridad alimentaria y de investigación biomédica, toxicológica y química. A diferencia de otras divisiones de las microalgas, la mayoría de los dinoflagelados poseen velocidades de crecimiento bajas. Una de las dificultades que presenta el cultivo de estos microorganismos es que los medios de cultivo que se emplean para microalgas utilizadas comúnmente en procesos industriales no son apropiados. Por tanto, para avanzar en la mejora de los procesos encaminados a la obtención de biomasa y bioactivos de dinoflagelados para distintos propósitos es necesario realizar esfuerzos en la reformulación de los medios de cultivo típicos. El diseño u optimización de medios mediante procedimientos experimentales clásicos es difícil, lento y costoso debido al gran número de nutrientes requeridos y a las posibles interacciones entre los componentes. La optimización mediante algoritmos genéticos (AG) es una técnica inteligente, aparentemente adecuada para sistemas biológicos poco definidos que requieren el manejo de un gran número de variables simultáneamente. El AG se desarrolla por bloques de experimentación, siendo el propio algoritmo el que marca la dirección de los cambios de un bloque a otro sin la participación del operador.

En este trabajo, se ha empleado un algoritmo genético (AG) para la optimización de la composición de un medio de cultivo adecuado para el dinoflagelado *Protoceratium reticulatum*. El procedimiento incluyó 26 componentes que fueron optimizados simultáneamente basándose en la productividad de biomasa. Tras 16 generaciones se halló una composición óptima que mejoró la concentración celular y la producción de YTXs totales en un 60% y 40%, respectivamente, en relación a cultivos control con el medio típico L1.

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P16. PRODUCTION OF OIL AS FEEDSTOCK FOR BIODIESEL PRODUCTION FROM HETEROTROPHIC FED-BATCH FERMENTATION OF *CHLORELLA PROTOTHECOIDES* USING GLYCEROL AS CARBON SOURCE

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If during the coming decades there is a worldwide huge increase in the production of biodiesel fuels, then the problem of efficiently treating wastes containing glycerol may possibly have similar dimensions, or higher, than those of sugar-rich residues. Since this problem will need to be addressed, the glycerol should perhaps be considered as a strategic fermentable sugar alcohol. An easy solution is the bioconversion of this one to new biodiesel in the same facility where it is produced.

Fermenters have the lowest production costs per litre of algae oil, accounting for 23% of the same the costs of the organic carbon substrate. In heterotrophic conditions (using organic carbon under dark conditions) microalgae are grown in fermenters. Algae growth is independent of light energy which allows for much simpler scale-up possibilities since smaller reactor surface to volume ratios may be used[1]. These systems provide a high degree of growth control and also lower harvesting costs due to the higher cell densities achieved[2]. Heterotrophic fermentation of the microalga Chlorella protothecoides in bioreactors for large-scale biodiesel production has been proved to be possible using glucose as carbon source, but not with glycerol[3,4].

In this work, fed-batch cultures with Chlorella protothecoides were carried out in a conventional 2-L glass stirred-tank bioreactor. Glycerol was used as the main source of carbon in the culture medium. The maximum values of biomass concentration and productivity observed were, respectively, 64 gL^{-1} and 9.4 $gL^{-1}day^{-1}$. The highest level of total lipids in the dry biomass was a 42 % (d.w.), the maximum saponifiable lipid content being 36 % (d.w.). The accumulation of lipids was only evident to the end of the time of culture, when the biomass concentration attained was high enough. The oleic acid was the most abundant fatty acid in the saponifiable lipid fraction with a content of approximately 60% (on f.a.). The total content of oleic acid, linoleic acid, and palmitic acids was around 89% (on f.a.). The average cetane number of the saponifiable lipids (51) and the corresponding average ester melting point (-17ºC) describe this oil as a feedstock undoubtedly appropriated for biodiesel production.

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[1] G.H. Huang, F. Chen, D. Wei, X.W. Zhang, G. Chen. *Appl. Energ.* **2010**. 7, 38-46.

[2] C-Y. Chen, K.-L. Yeh, R. Aisyah, D.-J. Lee, J.-S. Chang. *Bioresour. Technol.* **2011**. 102, 71-81.

[3] W. Xiong, X. Li, J. Xiang, Q. Wu. *Appl. Microbiol. Biotechnol.* **2008**. 78, 29-36.

[4] X.F. Li, H. Xu, Q.Y. Wu. *Biotechnol. and Bioeng.* **2007**. 98, 764–771.

P17. B-CELL HYBRIDOMA COCULTURE WITHIN EL-4 THYMOMA CELLS ENHANCES HYBRIDOMA CELL GROWTH AND MONOCLONAL ANTIBODY PRODUCTION

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In this study, we have investigated a possible helper activity by the murine thymoma cell line EL-4 in stimulating hybridoma cell growth (55-6 cell line) and monoclonal antibody (mAb) secretion in cultures containing both cell types.

Results obtained shown that the presence of EL-4 cells in conditions of low numbers of EL-4 cells per 55-6 cells (1 EL-4 cell per 4 55-6 cells) had a helper activity in stimulating the specific growth rate and mAb production by 55-6 cells. Both populations were seeded in co-culture without previous stimulation and, therefore low constitutive CD40L and CD40 expression levels and in the absence of exogenous co-stimulus. Regulation of CD40 and CD40L expression on the surface of 55-6 and EL-4 cells respectively in co-culture suggest interaction between both populations mediated by endogenous cytokines and co-stimulatory molecules. We also observed a positive effect of 55-6 cells on specific EL-4 growth rate. Contrary to what was observed for 55-6 cells, the increasing proportions of 55-6 cells per EL-4 cells did not decrease this effect suggesting that EL-4 cell growth is not limited by the nutrient competition between both cell types.

Therefore, we have established an *in vitro* B-cell hybridoma-T lymphocyte co-culture system closer to the in vivo cell-cell interaction between B and T lymphocytes and feasible to scale to a bioreactor to enhance proliferation and monoclonal antibody production in a cheaper way than other current processes.

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P18. EFFECTS OF CD40 ACTIVATION IN B-CELL HYBRIDOMAS ENGINEERED TO OVEREXPRESS CD40: CLONAL VARIATION FOR PROLIFERATION AND MONOCLONAL ANTIBODY PRODUCTION

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Vigorous growth of hybridomas and stable and high production of antibodies are the two major challenges faced by the hybridoma-based monoclonal antibody industries. Genetic modulation of CD40 dependent signaling pathways could be a route to achieve these goals of improved stability and production of antibodies. We present early results of studies of CD40 activation in human antibody-secreting B-cell hybridomas engineered to overexpress CD40. All hybridoma clones were from the same fusion event from a single mouse immunized with HIV-1 gp140 antigen. Three B-cell hybridoma clones overexpressing CD40, two of which clonally related, were activated using increasing concentrations of either CD40 ligand (CD40L) or anti-CD40 antibodies in the presence of interleukin (IL)-2, IL-4, IL-10 and lipopolysaccharide (LPS). The early results of our studies suggest clonal level differences in both proliferation and antibody production by hybridomas overexpressing CD40 and activated by either anti-CD40 or CD40L. Some clones responded positively to CD40 stimulation and showed greater proliferation, or greater antibody production. Others showed little or no effects and sometimes detrimental effects of CD40 activation. Our early results suggest that clones of hybridomas secreting antibodies to the HIV-1 gp140 antigen can be genetically engineered to overexpress CD40 and could provide a valuable resource for isolating sub-clones / cell lines with high proliferation in vitro and also higher production of antibodies. The stability of some of these hybridoma clones are currently being assessed and validated.

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P19. CONCENTRATION OF DOCOSAHEXAENOIC ACID (DHA) AND EICOSAPENTAENOIC ACID (EPA) BY SELECTIVE ALCOHOLYSIS CATALIZED BY LIPASES

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The aim of this work was to find acyl-selective lipases to catalyze the ethanolysis of tuna oil (22.7% DHA, 9.1% EPA) with the goal obtaining EPA or DHA rich acylglycerols. The acyl-specificities of different lipases were screened for concentrating DHA and EPA in the acylglycerols, because these lipids are the natural substrates for human food.

The activity and DHA or EPA specificity of the following commercial immobilized lipases were evaluated: LipozymeRM IM from *Rhizopus miehei* RM), LipozymeTL IM from *Thermomyces lanuginosus* TL), lipase QLC® and lipase QLG®, both from *Alcaligenes* sp., Novozym® 435 from *Candida antarctica* (N435), lipase DF® from *Rhizopus oryzae* (DF), and lipases from *Rhizopus oryzae* (RO), *Thermomyces lanuginosus (Th.L), Candida rugosa* and *Candida cylindracea*, last four immobilized on Immobead 150.

The alcoholysis reaction was carried out in an anhydrous solvent-free system, with low ethanol concentrations in order to (1) emphasize the comparison between the specificities of the different lipases and (2) to provide better stability to the lipases in the operational conditions.

The results show that lipases QLC® and QLG® are acyl-selective towards EPA and LipozymeTL IM, LipozymeRM IM and *Thermomyces lanuginosus* immobilized on Immobead 150 towards DHA. By using lipase QLC, EPA levels in the acylglycerol fraction increased almost two-fold, (from ~9 to ~16% by weight), at ethanolysis conversions around 55-57%, with EPA recovery yields over 90%. With Lipozyme TL IM DHA levels in the acylglycerol fraction increased almost two-fold again, (from ~23 to ~43% by weight), at ethanolysis conversions around 50-55%, with DHA recovery yields over 85%.

P20. CAPTACIÓN DE CO_2 MEDIANTE CULTIVO DE MICROALGAS A GRAN ESCALA EN REACTORES RACEWAY

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El CO_2 proveniente de la combustión de derivados del petróleo, gas y carbón, ha sido identificado como el principal factor implicado en el calentamiento global. De las distintas iniciativas que se han puesto en marcha para captar el CO_2, el cultivo de microalgas es una de las más prometedoras. Se trata de una tecnología de carácter renovable basada en el mecanismo natural de captura de CO_2: la fotosíntesis. Este trabajo de investigación está encaminado al diseño, desarrollo y puesta a punto de un sistema capaz de asimilar el carbono presente en el CO_2 y producir biomasa de microlgas con distintas aplicaciones: bioenergía, producción de proteínas y depuración de aguas residuales, entre otras. El sistema de cultivo elegido fue un fotobiorreactor de bajo coste tipo raceway (carrusel). Se trata de un canal de agua poco profundo en el cual el medio de cultivo circula mediante el uso de ruedas con aspas. La poca profundidad de los raceways favorece la alta exposición solar necesaria para el crecimiento de las microalgas.

La experimentación llevada a cabo en este trabajo se hizo en dos reactores raceways con una longitud total de 100 metros cada uno,anchura de canal de 1 metro y volumen máximo de 30 m3. Estos raceways tienen una capacidad de operación entre 0,1 y 0,3 m de lámina de agua. Para aumentar la transferencia del CO_2, el gas se burbujeo en un foso de 1 metro de profundidad lo que permite un mayor contacto entre las fases líquida y gaseosa. En el centro del foso se instaló un deflector que fuerza a todo el volumen de líquido a pasar por la zona próxima al burbujeador de gases.

El comportamiento fluidodinámico de los fotobiorreactores influye en el crecimiento de las microalgas y los fenómenos de transferencia gas/liquido. En uno de los raceways se realizó una caracterización fluidodinámica mediante pulsos de solución saturada de NaOH y registro de los valores de pH a lo largo del reactor. Estos experimentos se realizaron a distintas velocidades de líquido y con dos configuraciones de foso: con y sin deflector. Los parámetros utilizados para evaluar la fluidodinámica fueron: tiempo de mezcla, tiempo de ciclo y grado de mezcla (nº Bodestein, Bo). Se observó que la presencia del deflector influye de forma considerable aumentando los tiempos de ciclo y mezcla, al disminuir la velocidad de circulación del líquido. Sin embargo, el deflector aumentó considerablemente el grado de mezcla a similares velocidades de líquido. Así, la determinación del número de Bodenstein en cada tramo revela que la máxima mezcla se produce en las paletas y en el foso. En cambio, los tramos rectos presentan un Bo muy elevado correspondiente a un flujo ordenado tipo pistón, sin mezcla longitudinal. Las curvas del raceway presentaron una situación intermedia de grado de mezcla. Complementariamente, se registró el consumo energético del motor que mueve la rueda de aspas, detectándose valores muy superiores cuando el deflector estaba presente.

Otro de los raceways se usó para realizar ensayos preliminares de cultivos de microalgas. Se ha inoculado con la microalga *Scenedesmus*, manteniéndose estable durante más de 50 días, con concentraciones máximas 0,8 g/L, funcionando a un tiempo de residencia hidráulico de 6,5 días. La dificultad para alcanzar un estado estacionario se puso de manifiesto en diversas ocasiones en las que las microalgas se decantaron en el propio raceway, disminuyendo la eficiencia fotosintética. Dentro de este contexto, se esta trabajando en un sistema de medida de actividad fotosintética *in situ* como método para determinar el estado de las células a diferentes profundidades y detectar las zonas no activas del reactor en las capas más profundas. De esta forma, se trabajará para aumentar la actividad fotosintética optimizando la altura del nivel de líquido, velocidad de circulación y turbulencia del sistema, parámetros todos ellos relacionados entre sí como se observó en la caracterización fluidodinámica.

P21. EFECTO DE LA TEMPERATURA EN LA DESINFECCIÓN DE AGUAS RESIDUALES POR FOTO-FENTON

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Estudios recientes han demostrado la viabilidad de los procesos de oxidación avanzada (PPOA) como una alternativa viable a los tratamientos tradicionales de aguas residuales. Los PPOA están basados en la generación de especies fuertemente oxidantes y, entre ellos, tienen un especial interés aquellos que emplean la luz solar como fuente de energía, gratuita y respetuosa con el medioambiente. De entre todos, el proceso de foto-Fenton solar es el más indicado para la detoxificación de las aguas residuales contaminadas[1]. Esta tecnología también puede ser aplicada para la desinfección de las aguas residuales, puesto que, el alto potencial de oxidación de los radicales hidroxilo causa la inactivación de los microorganismos, aunque la información en este campo es escasa.

La desinfección por fotocatálisis solar es un proceso que tiene lugar a temperatura ambiente. La temperatura varía a lo largo del día y a lo largo del año con las estaciones en un rango aproximado entre 10 - 40ºC. Por ello, se hace muy interesante el estudio del efecto de la temperatura y su influencia en el proceso. Por tanto, el objetivo de este trabajo se centra en el estudio del efecto de la temperatura en la inactivación bacteriana por foto-Fenton teniendo en cuenta los principales parámetros (irradiancia, concentración de peróxido de hidrógeno y catalizador). Con este propósito, *Enterococcus faecalis*, modelo de microorganismo Gram-positivo, se seleccionó por su uso como indicador de contaminación fecal. Además esta especie ha sido muy poco estudiada en comparación con *Escherichia. Coli*. Agua contaminada con resorcinol[2] y *E. faecalis* se trató por foto-Fenton a cuatro temperaturas, 10, 20, 30 y 40ºC.

Para estudiar el efecto de la temperatura en la inactivación de *E. faecalis*, se comprobó, en primer lugar, el efecto aislado del estrés mecánico provocado por la bomba observando que este no afectaba a la viabilidad de las células. Tanto el efecto de la radiación UV como del peróxido de hidrógeno producen un descenso de 2-log, tardando 80 minutos en el primer caso y 120 minutos en el segundo. El efecto combinado de radiación UV y peróxido alcanza el límite de detección (1-log) en 120 minutos, mientras que el tratamiento mediante foto-Fenton alcanza este mismo límite en 80 minutos. Los datos confirmaron que foto-Fenton es la opción más eficiente frente a las demás, siendo por tanto un método eficaz para la inactivación de microorganismos característicos de las aguas residuales. En cuanto a la tasa de inactivación de *E. faecalis* se demostró que ésta es mayor al incrementar la temperatura. A 10ºC de temperatura no se alcanza el límite de detección, a 20ºC el límite de detección se alcanza a los 80 minutos, a 30ºC se tarda 65 minutos frente a los 40 minutos que se tarda a una temperatura de 40ºC. El estudio de los parámetros influyentes en el proceso es clave a la hora de ahorrar costes. Se observó que el aumento de la temperatura disminuye el tiempo de inactivación de *E. faecalis* lo que supone un dato clave a la hora de reducir el tiempo de reacción, que junto con el coste de reactivos, son los dos factores económicos más influyentes en el proceso[3].

Agradecimientos. Consejería de Innovación, Ciencia y Empresa de la Junta de Andalucía (FOTOMEM, P08-RNM-03772), Ministerio de Ciencia e Innovación (FOTOREG, CTQ2010-20740-C03-01/PPQ) y FEDER.

[1] M.M. Ballesteros, J.A. Sánchez, J.L. García, J.L. Casas, S. Malat. Effect of pesticide concentration on the degradation process by combined solar photo-Fenton and biological treatment. Water Research, Vol. 43, Issue 15 , **2009**, pp3838-384.8

[2] Dorothee Spuhler, Julian Andrés Rengifo-Herrera, César Pulgarin. The effect of Fe^{2+}, Fe^{3+}, H_2O_2 and the photo-Fenton reagent at near neutral pH on the solar disinfection (SODIS) at low temperatures of water containing *Escherichia coli* K12. Applied Catalysis B: Environmental, Vol. 96, Issues 1-2, **2010**, pp. 126-141.

[3] L. Santos-Juanes , M.M. Ballesteros, E. Ortega, A. Cabrera, I.M. Román, J.L. Casas, J.A. Sánchez. Economic evaluation of the photo-Fenton process. Mineralization level and reaction time: The keys for increasing plant efficiency. Journal of Hazardous Materials, Vol. 186, Issues 2-3, **2011**, pp 1924-1929.

P22. DEVELOPMENT OF A PROCESS FOR THE PRODUCTION OF L-AMINOACIDS CONCENTRATES FROM MICROALGAE BY ENZYMATIC HYDROLYSIS

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A process for the production of L-aminoacids concentrates from dry-freezed microalgae biomass by enzymatic hydrolysis has been developed. The process is composed of a pre-treatment for cell-disruption, an enzymatic hydrolysis and a final separation by centrifugation. Thermal and mechanical cell-disruption methods at different intensities have been tested and a mechanical disruption using bead milling for 30 minutes was found adequate. Longer times did not improve the yield of the process and the addition of a disintegrating agent such as alumina was unnecessary. The enzymatic hydrolysis was done in a two-step process using the enzymes Alcalase and Flavourzyme. Maximum yield was obtained for biomass concentrations under 270 g/l and previous additional treatment with Viscozyme, reaching a 42% hydrolysis. Repeated hydrolysis steps increased the yield from 42% (3 h processing time) with a single step to 59% (8 h) after two successive hydrolysis steps. Further increase of the number of hydrolisys steps had a meagre impact on the global yield. According to the data presented, an optimal process should include cell-disruption, Viscozyme treatment, two-step enzymatic hydrolysis and centrifugation to obtain the final product (Figure 1). This process widens the portfolio of products that can be obtained from microalgae biomass and is a new possibility to enhance the economic viability of microalgae-based biofuels production processes.

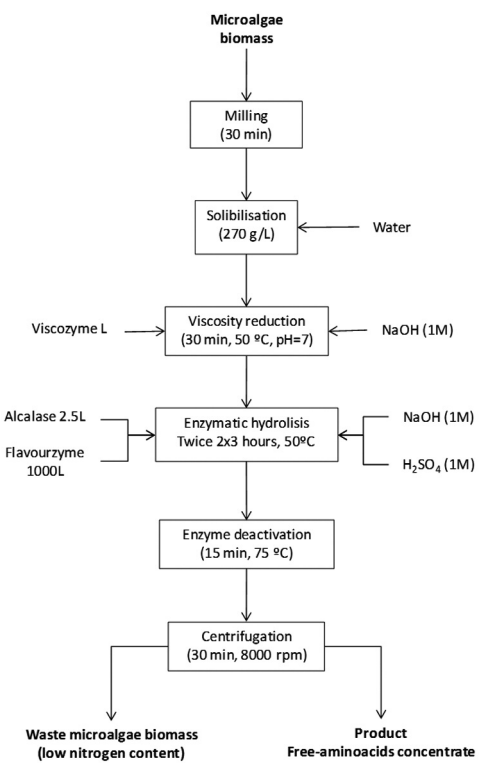

Figure 1. Block-diagram of the proposed process for the production of free-aminoacids concentrates from *Scenedesmus almeriensis* microalgae biomass.

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P23. REGIONAL CONTROLS OF ECOSYSTEM FUNCTIONAL TYPES RICHNESS IN TEMPERATE SOUTH AMERICA

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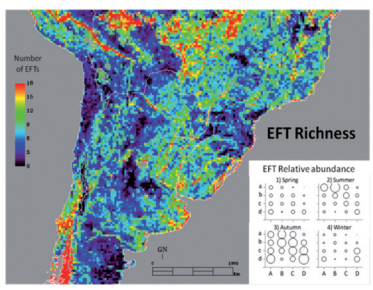
Global environmental change effects on biodiversity are particularly noticeable at the ecosystem level and have a faster influence on the functional than on the structural or compositional components. However, the regional controls of biodiversity patterns have been traditionally evaluated using structural and compositional components of biodiversity. During the last decades, though, the role of ecosystem functioning in both management and conservation is increasing. Approaches such as the remote sensing-based monitoring of Ecosystem Functional Types[1] (EFTs, patches of the land-surface with similar carbon gain dynamics) now open the possibility of characterizing over vast areas the spatial and temporal heterogeneity of functional diversity at the ecosystem level. Our aim was to evaluate whether the environmental controls of species richness that are globally observed can also be revealed at the ecosystem level using EFTs richness (Figure 1) across natural and agricultural systems in non-tropical South America. The yearly identification of EFTs was based on three descriptors of carbon gain dynamics derived from seasonal curves of Enhanced Vegetation Index (EVI): annual mean (surrogate of primary production), seasonal coefficient of variation (indicator of seasonality), and date of maximum NDVI (descriptor of phenology). The EVI timeseries was obtained from the MODIS-Terra satellite dataset (MOD13A1) and consisted in 16-day maximum value composite images at a spatial resolution of 0.05° x 0.05° from 2001 to 2008. The climate dataset (CRU TS3.10) consisted in monthly gridded time-series at a spatial resolution of 0.5º x 0.5º including precipitation, potential evapotranspiration, and temperature. Relief heterogeneity was derived from the SRTM DEM. Precipitation, Potential Evapotranspiration, Relief heterogeneity, and seasonality showed the largest single main effects on EFT Richness (Table 1). As observed for species richness in the southern hemisphere[2], not energy, but water availability, emerged as the main climatic driver of EFTs Richness patterns in temperate South America. Relief heterogeneity, and daily, seasonal, and interannual variability in climate also controlled EFTs Richness.

Table 1.

	Explained Deviance			Akaike Inform. Crit.		
	x	x^2	x^3	x	x^2	x^3
Precipitation	0.068	**0.102**	0.104	22455	**22320**	22313
Potential EvapoTransp.	**0.091**	**0.093**	0.095	22362	**22355**	22348
CV Altitude	**0.070**	**0.073**	0.073	22447	**22438**	22438
Seasonal CV PET	0.000	0.050	**0.061**	22732	22530	**22488**
Interannual CV PET	0.000	0.033	**0.057**	22731	22600	**22505**
Diurnal Thermic Range	0.046	0.046	**0.053**	22543	22545	**22522**
Seasonal CV DTR	0.032	**0.046**	0.046	22601	**22548**	22550
Temperature	0.008	0.018	**0.043**	22701	22662	**22562**

Figure 1.



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[1] Alcaraz-Segura D., JM. Paruelo, J. Cabello, Identification of current ecosystem functional types in the Iberian Peninsula, *Global Ecology and Biogeography* **2006**, *15*, 200–212.

[2] Hawkins, B. A., R. Field, H. V. Cornell, D. J. Currie, J. F. Guegan, D. M. Kaufman, J. T. Kerr, G. G. Mittelbach, T. Oberdorff, E. M. O'Brien, E. E. Porter, and J. R. G. Turner, Energy, water, and broad-scale geographic patterns of species richness, *Ecology* **2003**, *84*, 3105–3117.

P24. THE ROLE OF VEGETATION AND LITHOLOGY IN THE SPATIAL AND INTER-ANNUAL RESPONSE OF EVI TO CLIMATE IN DRYLANDS OF SOUTHEASTERN SPAIN

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The regional spatial and inter-annual response of the Enhanced Vegetation Index (EVI, as a proxy for aboveground net primary production) to environmental controls was evaluated in drylands of SE Spain. By means of linear mixed-effects models we found that both the spatial patterns and inter-annual trends of the EVI annual mean were explained by climate variability but clearly modulated by lithology and vegetation. Along the spatial gradient, precipitation increased the EVI mean even compensating for the greater evapotranspiration of warmer sites. Limestones, with high available water content, showed the lowest dependence of EVI mean on precipitation. The greater capacity of scrublands to store and use soil moisture was only evident on marls sites. The observed 2001-2010 trends towards less stressful conditions (precipitation rises and temperature declines) led to EVI mean increases. This EVI mean response was steeper in grasslands, with shallow roots, and marls, with low available water content. The study revealed the importance of analyzing the seasonal timing of trends in Mediterranean drylands, where temperature and precipitation are out of phase. The observed earlier rain-arrival after summer drought and cooler early-autumn, caused very strong EVI increases at the beginning of the growing season that may be favorable for the rest of the season.

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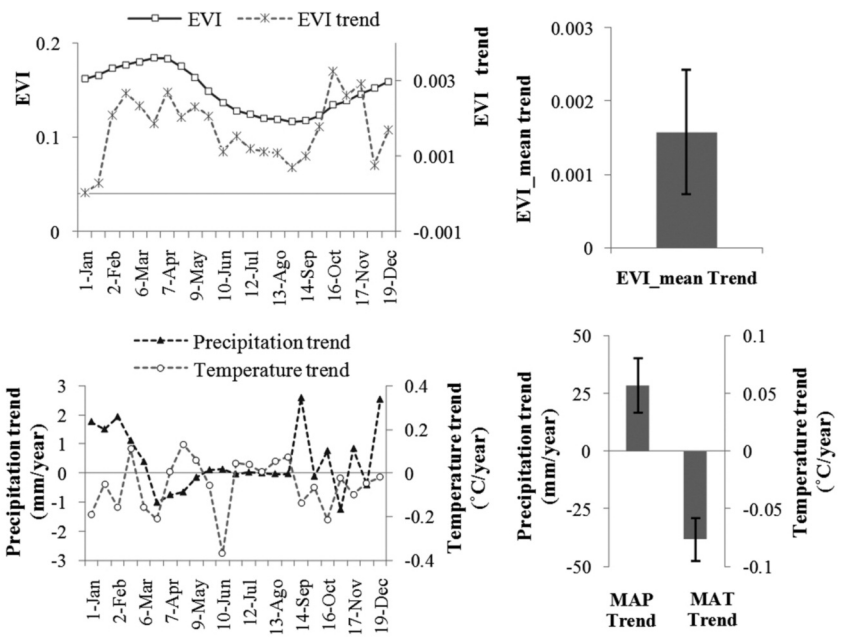

Figure 1. Inter-annual trends along the 2001-2010 study period for a) the seasonal EVI values (the mean seasonal EVI curve is also shown), b) the EVI annual mean (EVI_mean, a surrogate of primary production), c) seasonal temperature and precipitation values, and d) mean annual temperature (MAT) and mean annual precipitation (MAP) values. Y axes represent the average Sen's slope of 137 sampled pixels. For details see[1]

[1] J. Cabello, D. Alcaraz-Segura, R. Ferrero, A.J. Castro, E. Liras, *Journal of Arid Environments* **2011** in press.

P25. THE ROLE OF BIOLOGICAL SOIL CRUSTS ON RUNOFF, EROSION AND FERTILITY IN DRYLANDS

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Deserts and semiarid ecosystems (shrublands and grasslands) are the largest terrestrial biome, covering more than 40% of the Earth's terrestrial surface. In these ecosystems, the open areas between vascular plants are usually covered by a community formed by cyanobacteria, fungi, algae, lichens, mosses and liverworts, which is known as biological soil crusts (BSCs) and that can cover up to or more than 70% of the soil surface[1]. Biological soil crusts affect multiple soil properties that are associated to key ecosystem processes in aridlands such as hydrological processes or nutrient cycling[2]. They have been described as ecosystem engineers in aridlands since they: i) have a strong influence on soil porosity, cracking, roughness or hydrophobicity, thus affecting infiltration and runoff, evaporation and soil moisture[1], ii) secrete polysaccharides that adhere soil particles together and increase soil aggregation and stability, thereby reducing erosion by water and wind[3], iii) fix atmospheric carbon and nitrogen and are capable of photosynthesis, respiration and of decomposing and mineralizing organic compounds, so they strongly affect nutrient cycling and represent major sources of carbon and nitrogen in arid ecosystems[4], and iiii) affect the germination, emergence and survival of vascular plants through changes in soil properties[5]. Collectively, BSCs perform vital ecosystem services; however the rate and type of these services vary depending on the species abundance and crust composition[6]. When crust organisms colonize the soil, they spread out until occupying large extents of the soil surface and later the development continues by replacing one species by others. Cyanobacterial crusts represent early successional stages of BSCs, whereas lichens and mosses appear during late stages of BSC development[7].

The aim of this work was to examine the influence of different types of BSCs on runoff and erosion and on soil carbon and nitrogen contents in two semiarid ecosystems in the province of Almeria where soil crusts are well-represented: El Cautivo (Tabernas desert) and Amoladeras (Cabo de Gata-Níjar). The influence of BSCs on runoff and erosion was analyzed by rainfall simulation and under natural rainfall in small plots set up in the field. Samples of the different crust types, as well as of their underlying soils (0-1 cm and 1-5 cm soil layers under the crusts) were collected and organic carbon and nitrogen contents were determined in the laboratory. We found that runoff and erosion decreased with greater crust development, that is, as cover and biomass of crust organisms increased and later successional species as lichens and mosses colonized the crust. Organic carbon and total nitrogen significantly increased in the crust and its underlying soil, especially in the top 1cm-soil underneath the crust, as the development of the crust was higher. Therefore, our results highlight the relevant role of BSCs in water availability and fertility in soils of semiarid areas.

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[1] J. Belnap, *Hydrol. Process.* **2006**, *20*, 3159-3178.

[2] F.T. Maestre, M.A. Bowker, Y. Cantón, A.P. Castillo-Monroy, J. Cortina, C. Escolar, A. Escudero, R. Lázaro, I. Martínez, *J. Arid. Environ.* **2011**, 75, 1282-1291.

[3] G. Mazor, G.J. Kidron, A. Vanshak, A. Abeliovich, *FEMS Microbiol. Ecol.* **1996**, *21*, 121-130.

[4] D.M. Mager, A.D.Thomas, *J. Arid. Environ.* **2011**, *75*, 91-97.

[5] D.J. Eldridge, R.S.B. Greene, *J. Arid. Environ.* **1994**, *26*, 221-232.

[6] D.C.Housman, H.H. Powers, A.D. Collins, J. Belnap, *J. Arid. Environ.* **2006**, *66*, 620-634.

[7] R. Lázaro, Y. Cantón, A. Solé–Benet, J. Bevan, R. Alexander, L.G. Sancho, J. Puigdefábregas, *Geomorphology* **2008**, *102*, 252-266.

P26. BIODIVERSIDAD EN SISTEMAS ACUÁTICOS ARTIFICIALES: EL CASO DE LAS BALSAS

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Las balsas, como ejemplo de socio-ecosistema, constituyen sistemas acuáticos artificiales creados para satisfacer las demandas de agua. En 2007, la Consejería de Medio Ambiente censó un total de 16543 balsas, la mayoría de ellas con usos agrícolas y agro-ganaderos. En conjunto, estas balsas mostraron gran heterogeneidad respecto a su localización geográfica, tipos constructivos, tamaño, origen del agua, estado de naturalización y prácticas de gestión[1], y suponen nuevos hábitats para los organismos acuáticos[2]. Los resultados del muestreo y análisis de una selección de estas balsas distribuidas por toda Andalucía revelan una gran diversidad regional: 12 especies de anfibios, 11 de peces, 4 de reptiles, 68 familias de macroinvertebrados, 38 especies de vegetación acuática sumergida, 103 especies de zooplancton y 293 de fitoplancton. Sin embargo, el papel de estos sistemas en la conservación de la diversidad depende en gran medida de sus características[3], de modo que las balsas más diversas presentan sustrato natural con pendientes suaves, vegetación acuática y niveles tróficos intermedios. Entre las principales amenazas que pueden mermar la biodiversidad de las balsas destacan la presencia de especies invasoras, como la gambusina y el cangrejo rojo americano, la eutrofización y las técnicas de manejo agresivas, como la adición de biocidas. A la luz de nuestros resultados, podemos proponer algunas recomendaciones para compatibilizar el uso eficiente de la balsa con la conservación de la biodiversidad, entre las que destacan el mantenimiento de la vegetación acuática sumergida, evitar el uso de biocidas y optar por diseños constructivos con sustratos naturales o revestimiento de grava, en caso de sustratos artificiales.

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[1] J.J. Casas, J. Toja, S. Bonachela, F. Fuentes, I. Gallego, M. Juan, D. León, P. Peñalver, C. Pérez, P. Sánchez, *Water Environ. J.* **2011***, 25*, 308-317.

[2] P. Williams, M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet, D. Sear, *Biol. Conserv.* **2004***, 115*, 329-341.

[3] S. Lutton, F. Sheldon, S.E. Bunn, *Aquatic Conserv: Mar. Freshw. Ecosyst.* **2010***, 20*, 47-57.

P27. EFFECTIVENESS OF AMENDMENTS IN REMEDIATION OF A METAL-ARSENIC POLLUTED SOIL USING *Lactuca sativa* L. BIOASSAYS

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A metal-arsenic polluted soil from sulphide-mine waste was treated, in all possible combinations, with two different amounts of marble sludge (MS), compost (CM), and Byferrox (BF). These amendments were mainly composed by calcium carbonate, organic carbon, and iron oxide, widely used in soil remediation[1,2]. In order to evaluate the effectiveness of amendments in the trace-elements immobilization, bioassays using different development stages in lettuce (*Lactuca sativa* L.) were performed[3]. Firstly, lixiviate and pore water from each treated and untreated soil (27 treatments) were analysed, and seed germination bioassays were performed (germination index-GI and root elongation index-REI were measured). The best amendments combination (9 treatments) in decrease trace element concentration in pore water, and reduce toxicity for lettuce germination, were selected for emergence and establishment bioassays. Plastic pots were filled with 150 g of uncontaminated natural soil (soil-control), contaminated soil without amendments, and amended soils. After two week, emergence (EI) and proportional elongation of the plants respect to the soil control (PEI) index were measured. After 14 weeks, established lettuces were carefully removed from the pots and the proportional dry weight respect to the control (PDW), and foliar trace elements concentration were measured. Soils after bioassays were analyzed using a sequential extraction procedure[4].

Marble sludge and compost increased the pH values and decreased the Zn, Cd, Cu, and Pb concentrations in lixiviates and pore water. Germination bioassays showed that all lixiviates were highly phytotoxic and seeds did not germinate. All seeds germinated in pore water from amended soil, and root elongation index (REI) showed significant differences between treatments. Toxicity in pore water was mainly decreasing by marble sludge application. Emergence index (EI) was higher in amended soil respect to unamended soil, but significant differences between treatments were only found for proportional elongation index (PEI)., showing that high REI values does not necessarily imply greater plant elongation at later stages of development. Only lettuces sowed in soil amended with higher proportion of compost (6%) were established, with significant differences for plant length and weight. Foliar concentration was higher in lettuce cultivated in amended soils than in control lettuces (cultivated in a natural no contaminated soil). Trace element concentrations in plant varied between treatments, showing that combination of three amendments at higher rate of application was the most effective to reduce bioavailability. The main metal fractions involved in metal uptake were; a) acetic acid extracted fraction, which was directly related with foliar concentration; b) hydroxylamine and hydrogen peroxide extracted fractions, which were both inversely related with foliar concentration. Our results suggest that plants, in order to uptake nutrients from soil, can alter the chemical environment of the rhizosphere to stimulate desorption of ions from soil solids into solution; and trace elements previously immobilized by amendments can be bioavailable and causing toxicity to the plants.

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[1] R.Ciccu , M. Ghiani, A. Serci, S. Fadda, R. Peretti, and A. Zucca, *Minerals Eng.* **2003**, 16, 187–192.

[2] F. del Moral, V. González, I. García, J.A.. Sánchez, S. de Haro and M. Simón, Fresenius Environmental Bulletin. **2010**, Vol. 19 – No 9b, 2071-2076

[3] EPA, United States Enviromental Protection Agency, Ecological Effects Test Guidelines, **1996**. Available from: www.epa.org

[4] A. Tessier, P. G. C. Campbell and M.Bisson, Analytical *Chemistry,* **1979***, 51*, 844–851

P28. WHY GYPSUM OUTCROPS MAINTAIN HIGH VEGETATION GREENNESS DURING THE SUMMER IN SEMIARID MEDITERRANEAN CLIMATES?

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Gypsum soils are confined to arid and semi-arid climates where low precipitation prevents gypsum from being removed by leaching. These substrates represent stressful conditions for vegetation, leading to a high specialization in plant species. By using satellite-derived vegetation index, we have observed that Iberian South East gypsum outcrops maintain during the summer, high vegetation greenness compared with other vegetation types that do not grow on gypsum. Several hypotheses can explain this behavior. Gypsum rocks can reduce evaporation because of the high reflexion/reflectance of solar radiation. The existence of biological and physical crusts can also reduce evaporation. In addition, some physical properties of gypsum rocks would change water soil availability with respect to seasonal patterns of precipitation by absorbing atmospheric water or smoothing water loss. The objective of this work is to study the difference in vegetation greenness between gypsum outcrops and others vegetation types from Iberian SE territory. Remote sensing, radiometry, and field work will be used to test the former hypotheses. MODIS-TERRA satellite images from MODQ13Q1 product (composites of 16 days, 250 m of spacial resolution, from 2001 to 2009) will be used to calculate EVI, albedo and superficial temperature. Some local properties of gypsum outcrops related to geological properties and distance to the sea will be taking into account. The discussion of this work will be complemented with the radiometry and survey data.

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P29. SIDERÓFOROS EN TÉS DE COMPOST

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Los sideróforos son compuestos producidos por microorganismos del suelo que basan su actividad en fenómenos de quelación, un fenómerno rutinario en los sistemas biológicos. Los sideróforos son moléculas de bajo peso molecular que presentan grupos funcionales con elevada afinidad y especificidad por los iones ferrosos. Estos necesitan de receptores en la membrana celular de las bacterias para poder llevar a cabo su función. Los complejos hierro-sideróforos se unen a receptores proteicos de la membrana bac¬teriana externa, para ser transportados al espacio periplásmico por el complejo transperiplásmico.

En este trabajo se presenta la capacidad de producción de sideróforos de extractos acuosos (té) de compost de restos hortícolas, orujo de vid, vermicompost y compost de sustrato agotado procedente de la producción de champiñón. Los tés fueron preparados a 1/3 y 1/4 (p/v) en agua, y se incubaron durante 1, 3, 7 y 15 días, tanto en agitación orbital a 150 rpm (condiciones aireadas) o en oscuridad y reposo (condiciones no aireadas). La determinación de producción de sideróforos se realizó mediante el procedimiento seguido por Schwyn y and Neilands, (1987)[1]. Se realizó la cuantificación de sideróforos a los té filtrados, microfiltrados y esterilizados en autoclave a 120ºC durante 30 min.

Los resultados revelan una elevada producción de sideróforos por parte de la microbiota presente en los tés obtenidos de los cuatro compost analizados y en todas las condiciones ensayadas exceptuando, los microfiltrados donde no se observa ningún halo de decoloración correspondiente a la presencia de sideróforos.

[1] Schwyn, B. and Neilands, J.B. 1987. Universal Chemical Assay for the Detection and Determination of Siderophores. Analytical Biochemistry 160: 47-56.

P30. CRECIMIENTO DE CRISTALES DE ÇaCO$_3$, COMO RESULTADO DE LA ACTIVIDAD MICROBIOLÓGICA EN SUELOS

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Esta contribución se centra en los efectos que tiene un nuevo tratamiento microbiológico sobre las propiedades geotécnicas de los suelos, aplicado a la construcción de obras lineales. Con dicho propósito, diferentes suelos han sido tratados, en la universidad de Almería, con un producto de base microbiológica para mejorar sus propiedades mecánicas, basándose en la capacidad que tienen algunos microorganismos para precipitar sustancias con características cementantes, como el carbonato cálcico. Sobre este punto se presentan los resultados mineralógicos, químicos y estructurales obtenidos para una muestra de suelo areno limo-arcilloso (suelo natural B-5), sobre el que se lleva a cabo un tratamiento microbiológico (suelo tratado BT-5) y en el que se estudia el crecimiento de cristales de $CaCO_3$, para un posterior uso en obras de ingeniería. Para llevar a cabo el estudio del crecimiento de cristales, se han desarrollado diferentes ensayos de caracterización mineralógica, mediante Microscopio Electrónico de Barrido (SEM), junto con análisis químico por Espectroscopia de Energías Dispersivas de Rayos X (EDX), así como difracción de Rayos X (XRD). Algunas muestras se contrastan con resultados obtenidos en el microscopio óptico. También se realiza un estudio de las propiedades microestructurales del suelo, antes y después del tratamiento microbiológico, mediante Porosimetría por Intrusión de Mercurio (MIP).

El análisis mineralógico y químico del suelo B-5, mostró la predominancia de Silicio y Aluminio, lo que se asocia al cuarzo libre identificado por DRX, a la Albita y a la Microclina. Sin embargo, para el suelo tratado BT-5, se detectó además calcio, asociado con diferentes polimorfos del $CaCO_3$. Las imágenes de los minerales de carbonato obtenidas a través de SEM, se contrastan con un análisis cualitativo de EDX, que pone de manifiesto la aparición de cristales de $CaCO_3$ en el suelo BT-5, no habiéndose detectado con anterioridad en el suelo natural B-5. Se observa un crecimiento de los cristales en el área localizada en torno a los microorganismos como consecuencia de la sobresaturación de carbonatos y alcalinización del medio, condición necesaria para que el $CaCO_3$ precipite. Es posible que esta alcalinización del medio se deba al movimiento de los cationes de calcio entre la membrana celular de la bacteria y su entorno[1]. Como consecuencia de los diferentes procesos de precipitación química, dependientes de la sobresaturación de carbonatos, se observan diferentes estructuras cristalinas que actuarán de forma diferente sobre las características mecánicas del suelo[2]. En cuanto a las propiedades microestructurales, la comparación entre los datos obtenidos para las muestras B-5 y BT-5, muestran que el efecto de las bacterias se hace patente con una disminución de la porosidad en el rango 3μm – 30μm.

Además, la energía de compactación es menos efectiva reduciendo los poros de mayor tamaño[3]. Se puede decir que la precipitación de cristales de carbonato cálcico, tiene lugar en el espacio poroso del suelo, que presenta un tamaño superior a las dimensiones de los microorganismos (1μm-2μm). Es decir, los cristales precipitados actúan de material de relleno, sellando determinados poros. Como consecuencia, la función de distribución del tamaño de los poros cambia para el rango de 3μm-30μm, de forma que en el suelo tratado tiende a desaparecer o ser más pequeño. El uso de estos microorganismos como herramienta biotecnológica en el campo de la estabilización de suelos, se abre paso como posible cementante natural o como material de relleno del espacio poroso del suelo, de forma que puede variar las propiedades hidro-mecánicas de éstos.

[1] F. Hammes, W. Verstraete. Key roles of pH and calcium metabolism in microbial carbonate precipitation. Environ. Sci. Biotechnol. **2002**, 1, 3–7.

[2] S. Al-Thawadi. High strength in-situ biocementation of soil by precipitating locally isolated ureolytic bacteria. 2008. Tesis doctoral. Murdoch University.

[3] L. Morales, E. Garzón, E. Romero, C. Jommi. Effects of a microbiological compound for the stabilisation of compacted soils on their microstructure and hydro-mechanical behavior. 2010. Conference of Unsaturated Soils. Edited by Alonso & Gens.

P31. RESPUESTA HIDROLOGICA Y EROSIVA A LARGO PLAZO DE UNA CUENCA DE CÁRCAVAS. PREDICCIONES ANTE EL CAMBIO CLIMÁTICO

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Los sistemas acarcavados se consideran laboratorios geomorfológicos ideales para el estudio de los procesos erosivos, ya que estos ocurren a escalas espaciales y temporales mucho menores que en otros sistemas[1], lo que facilita su estudio. Estos sistemas se caracterizan por presentar elevadas tasas de escorrentía y erosión que generan importantes impactos aguas abajo (escorrentías salinas y cargadas de sedimentos) con implicaciones ambientales y económicas. Por lo tanto, es necesario conocer su comportamiento frente a futuros escenarios de cambio climático para predecir y poder controlar sus efectos. Estos escenarios apuntan una disminución en el volumen de precipitación y una variación en su distribución, concentrandose en unos pocos eventos de gran magnitud. Esta variación en el régimen de precipitación afectará a la escorrentía y a la erosión. Algunos autores han encontrado relaciones significativas entre la precipitación total y las tasas de erosión hídrica en sistemas de cárcavas, que usan como argumento para augurar una reducción de la erosión como consecuencia de la disminución de la precipitación. Sin embargo, a escala de cuenca interactúan diferentes procesos de deposición, transporte y erosión que pueden hacer que desaparezca ésta dependencia de la erosión de la cantidad de precipitación observada a pequeñas escalas. Para realizar un correcto manejo de las áreas acarcavadas y predecir los impactos de régimenes hidroclimáticos alterados, el primer paso es conocer su respuesta bajo condiciones de fuerte variabilidad inter-anual de la lluvia, como ocurre en el SE de España. Este trabajo analiza la influencia que la variabilidad en la precipitación ejerce sobre la respuesta hidrológica y erosiva en una subcuenca acarcavada situada en la cuenca de Sorbas-Tabernas. Para ello, se han analizado datos de escorrentía, precipitación y erosión recogidos desde 1991 en el área experimental de El Cautivo. La escorrentía se ha medido en un aforador flume tipo H, ubicado a la salida de la cuenca y equipado con una sonda capacitiva que registra la altura de la lámina de agua cada 20 segundos y un sistema de recogida automática de muestras para determinar la cantidad de sedimentos exportados en suspensión. Los sedimentos de mayor tamaño son recogidos en un colector situado a la salida del aforador.

Nuestros resultados muestran que la precipitación anual varía entre 112mmy^{-1} y 417mmy^{-1}, con una media de 216 mmy^{-1} para el periodo de estudio. Sólo un 20 % de los eventos generan escorrentía. La respuesta hidrológica de la cuenca es muy variable, oscilando entre 0.2 y 16 mmy^{-1} con una media anual de 4.8 mm•y^{-1}. La escorrentía registrada representa sólo un 2 % de la precipitación total. La producción de sedimentos varía entre 0.15 y 10.6 t•ha^{-1}•y^{-1}. Las tasas erosivas son menores de lo que cabría esperar para este tipo de sistemas y se explica por el efecto protector que ejerce la presencia de costras biológicas del suelo y a su distribución en el espacio[2]. La respuesta erosiva del sistema está controlada por la ocurrencia de eventos lluviosos intensos capaces de transportar el material erosionado fuera de la cuenca y no por la cantidad total de precipitación, como sugieren otros autores. Por este motivo podemos predecir un aumento de la erosión debido a la concentración de la precipitación en pocos eventos de gran magnitud.

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[1] A. D. Howard, **1994**. In *"Geomorphology of desert environments"*, A. D. Abrahams, y A. J. Parsons (Eds). London: Chapman and Hall (UK). pp 213-242.

[2] Y. Cantón, F. Domingo,A. Solé-Benet,J. **2001**, *252 (1-4), 65-84*.

P32. CAMBIOS ESPACIO-TEMPORALES EN EL ESTADO ECOLÓGICO DE LOS RÍOS AGUAS Y ALMANZORA: CONTRASTE DE ÍNDICES BIÓTICOS BASADOS EN MACROINVERTEBRADOS

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Desde hace años las metodologías para el estudio y seguimiento de la calidad de las aguas epicontinentales se han basado en análisis físico-químicos. El actual aumento de contaminantes, desde la contaminación difusa debida a las aguas residuales a principios del siglo pasado hasta los recientes relacionados con el Cambio Global y su efecto en el tiempo, han supuesto la adopción de índices bióticos mediante *bioindicadores*. La Directiva Marco de Aguas (DMA, 200/60/D.O.C.E., 2000) supone el punto de partida comunitario, y la transposición de ésta en España a través del Plan Hidrológico Nacional (PHN, 2001), establece la necesidad de evaluar no sólo la calidad biológica de los ríos, sino exige conocer la estructura y funcionamiento de los ecosistemas y su papel como proveedores de bienes y servicios.

Desde la transposición del IBMWP (antes 'BMWP) hasta los índices multimétricos cualitativos y cuantitativos utilizados actualmente, se han tratado de buscar aquellos índices bióticos que mejor representen el estado ecológico y salud de los ríos mediterráneos. Por ello, el objetivo de este estudio se ha basado en la determinación de los cambios espacio-temporales sufridos en el estado ecológico de los ríos Aguas y Almanzora a lo largo de 10 años, tomando como referencia el año 2000 (Proyecto Guadalmed) y el año 2010 (Proyecto Glocharid) mediante la utilización de índices bióticos basados en *macroinvertebrados*. Además se ha contrastado la bondad de estos índices para evaluar el estado ecológico en los ríos del sureste español.

Para la realización del mismo, ha sido necesario el análisis y muestreo de 25 localidades correspondientes a las cuencas del Río Aguas y Almanzora, y la determinación de los valores medios de las principales características físico-químicas: Temperatura, Ph, Conductividad eléctrica, Alcalinidad, OD, S.S., S.S.L.C.; concentración de nutrientes: $N\text{-}N0_3$, $N\text{-}NO_2$, $N\text{-}NH_4$, DIN, $P\text{-}PO_4$; morfológicas: Caudal, altitud, pendiente, anchura del cauce, longitud, y permanencia de la lámina de agua, además del índice de hábitat fluvial (IHF), el índice de vegetación de ribera (QBR) y el índice de impactos físicos (IIF) (Casas *et al.*, 2007). Además mediante muestreos semicuantitativos de 20 kicks (2,5 m^2) (Protocolo AQEM[1]) del zoobentos en las localidades de estudio, se obtuvieron las fracciones que fueron examinadas y sorteadas para la captura e identificación de los macroinvertebrados existentes en las mismas. Con los datos obtenidos para todas las localidades fueron calculadas distintas métricas como el IBMWP e IASPT, así como los índices multimétricos cualitativos: ICM-9 (ICM-Med) (Buffagni *et* Erba, 2006), ICM-11a (IMMi-L) (Munné *et* Prat, 2009) y cuantitativos: ICM-Star (Buffagni *et* Erba, 2006), ICM-7 (Buffagni *et* Erba, 2006), ICM-10 (IMMi-T) (Munné *et* Prat, 2009).

Como resultado de los análisis estadísticos (principalmente ACP, análisis de correlación y de regresión simple) se denota la existencia de un *Factor de impactos: Índice de impactos antrópicos* que se relaciona directamente con todas las variables físico-químicas, incluido el IIF sobre el canal y de manera inversa con el IHF y el QBR para ambos proyectos, además de con el caudal para el Glocharid. La inclusión del caudal para el Glocharid en el citado Factor de impactos pone de manifiesto la importancia de esta variable en la recuperación del estado ecológico para todas las localidades estudiadas a lo largo de 10 años, debido principalmente al aumento de las precipitaciones registrado para el año 2010. Sin embargo en algunas localidades

[1] **AQEM** (Assessment System for the Ecological Quality of Streams and Rivers).

el aumento de las precipitaciones no ha sido el factor determinante en la evolución del estado ecológico de los ecosistemas fluviales. Destacar localidades como Olula del Río donde la puesta en funcionamiento de un Estación Depuradora de Aguas Residuales ha mejorado el estado global, puesto de manifiesto en el aumento considerable de los índices bióticos, o el empeoramiento de localidades como Los Perales y Alfaix, motivado por impactos antrópicos como el tráfico rodado de vehículos o las obras ferroviarias del AVE. Para el caso de la Desembocadura de Mojácar la alta presión turística en el periodo estival, el vertido procedente de una estación de áridos cercana, el lixiviado de nutrientes procedente de la actividad agrícola comarcal, el efecto de la intrusión marina así como el comportamiento de esta localidad como un humedal ha supuesto el empeoramiento de la misma. Destacar además la elevada bondad que presenta el IBMWP frente al resto índices multimétricos estudiados, que denota diversas ventajas por la sencillez, facilidad y rapidez que supone su utilización.

P33. AN HYDRO-SPELEOLOGICAL APPROACH TO THE STUDY OF MINING IMPACT ON THE GYPSUM KARST OF SORBAS (SE SPAIN)

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The massif of Sorbas (Almeria, SE Spain) is one of the most karstified gypsiferous area of the world, with hundreds of dolines and different karst features. Its Messinian gypsum is composed of very pure selenite and has been exploited since 40 years ago. It is extracted from three large quarries, producing more than 200,000 tonnes/years of high quality gypsum. Although situated at the boundary of the Protected Natural Space of the Gypsum Karst of Sorbas, the exploitation has intensively affected the caves and their ecosystem, especially the landscape and the surface and underground water drainage. Before the complete impoverishment of such natural treasure, the harmonization between protection of the karst environment and mining activity is necessary. To achieve this goal, a karst index for detecting the most vulnerable areas has been implemented through measurements designed as part of a new adaptive management.

The study took place in the southern part of Sorbas gypsum plateau with several steps in multiple field-works for data collection, in each one proceeding with the measurement of the direction and dip of fractures and the inventory of different karst forms. Also water samples from the main springs have been collected for hydrochemical investigation purpose.

All field records are inventoried with corresponding spatial position and compared with the previously existing information. Subsequently, an exhaustive analysis of the data was performed leading to the definition of the karst features of the area, rendering by maps. To assess the extent of karstification, the spatial analysis of the distribution of the different cave entrances have been studied, discerning a possible indicator of the effects of gypsum mining.

This geospeleological index has been used to estimate the useful conditions of exploitation, enabling the conservation of most of the cavities, the preservation of the recharge basin of the karst system and springs, the minimum affection to the vadose groundwater flow, the protection of nearby springs and minimizing the visual impact.

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P34. LINEAR MAPS STRONGLY PRESERVING MOORE-PENROSE INVERTIBILITY

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Let A and B be C*-algebras. We say that an element a in A is Moore-Penrose invertible if there exists b in A such that

$$aba=a, \quad bab=b, \quad (ab)^*=ab \quad \text{and} \quad (ba)^*=ba$$

In this case, b is called the (unique) Moore-Penrose inverse of a and it is denoted by a^+. Given a linear map $T: A\to B$, we say that T strongly preserves Moore-Penrose invertibility if $T(a^+)=T(a)^+$ for every Moore-Penrose invertible element a in A.

We investigate linear maps from A to B strongly preserving Moore-Penrose invertibility where A is unital and either it is linearly spanned by its projections, or has real rank zero, or has large socle[1].

[1] M. Burgos, A. C. Márquez-García, A. Morales-Campoy, *Linear maps strongly preserving Moore-Penrose invertibility*, to appear in Operators and Matrices.

P35. ACELERACIÓN DEL MÉTODO DEL GRADIENTE BICONJUGADO PARA MATRICES DISPERSAS EN GPUS

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En una gran variedad de aplicaciones de diferentes disciplinas científicas y relacionadas con la ingeniería, se requiere la resolución de sistemas de ecuaciones no simétricos y complejos. Para resolver este tipo de sistemas de ecuaciones lineales, el Método del Gradiente Biconjugado (BCG) se considera especialmente relevante, ya que es capaz de resolver sistemas de ecuaciones complejos y no simétricos con una gran precisión. Sin embargo, desde el punto de vista computacional, dicho método presenta un alto coste computacional debido a los productos matriz dispersa vector (SpMV) involucrados.

Por lo tanto, para acelerar dicho método es necesaria la explotación de Computación de Altas Prestaciones (HPC). La computación GPU ha emergido como una nueva técnica de HPC que ofrece un paralelismo masivo y, por tanto, puede ser considerada como una valiosa herramienta para acelerar este tipo de algoritmos. En este trabajo se muestra que el BCG puede ser acelerado de una forma eficiente si todas las operaciones se computan en la GPU. Hemos considerado dos implementaciones distintas del método BCG en la GPU: $CuBCG_{SP}$ (basado en la librería CUSPARSE[1] desarrollada por NVIDIA) y $CuBCG_{ET}$ (basado en la rutina ELLR-T[2] desarrollada por nuestro grupo de investigación). Ambos desarrollos han sido evaluados para dos conjuntos de matrices de testeo de tipo complejo y real, en simple precisión. Los resultados experimentales han mostrado que ambas implementaciones, $CuBCG_{SP}$ y $CuBCG_{ET}$, obtienen rendimientos superiores a la implementación con múltiples cores del BCG en un computador multicore actual (8 cores). Destacando la implementación $CuBCG_{ET}$, que obtiene mejores factores de aceleración especialmente para el conjunto de matrices de tipo complejo (hasta 12x).

Tabla 1. Factor de aceleración de las matrices reales para los métodos $CuBCG_{CS}$ y $CuBCG_{ET}$ frente a la versión multicore (8 cores) del BCG utilizando la librería MKL.

Matriz Real	*$CuBCG_{CS}$*	*$CuBCG_{ET}$*
wbp128	3,48	4,44
cant	1,76	2,40
pdb1HYS	3,43	4,78
consph	1,89	2,29
shipsec1	3,12	3,99
pwtk	1,68	2,62
wbp256	4,09	4,41

Tabla 2. Factor de aceleración de las matrices complejas para los métodos $CuBCG_{CS}$ y $CuBCG_{ET}$ frente a la versión multicore (8 cores) del BCG utilizando la librería MKL.

Matriz Compleja	*CuBCGCS*	*CuBCGET*
kim1	1,03	1,95
femfilter	1,38	3,16
NN70	4,94	8,88
NN80	5,07	9,27
NN90	3,52	11,60
kim2	1,90	6,92
femhifreqcircuit	2,93	7,25

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[1] NVIDIA, *CUDA Cusparse library*, Tech. Rep., September. **2010**.

[2] F. Vázquez, G. Ortega, J.J. Fernández, E.M. Garzón, *Improving the performance of the sparse matrix vector product with GPUs*. 10th IEEE International Conference on Computer and Information Technology. CIT 2010. pp. 1146–1151. IEEE Computer Society. **2010**.

P36. CORNEAL TOPOGRAPHY RECONSTRUCTION BY GENERALIZED RADIAL BASIS FUNCTIONS

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An accurate fitting of the corneal surface is important for different reasons, among them the design of ocular contact lens, simulation of the real vision of a patient, analysis of the cornea for the laser surgery, detection and measurement of corneal irregularities and others.

Zernike polynomials are a well-known and established tool for modal reconstruction of the cornea from the height or curvature data. Standard RBF functions have been presented in the literature previously[1] for the same purpose, yielding good results in comparison with the Zernike fit. We discuss two alternative approaches, both based on a generalization of the radial basis functions (RBF) that combine the simplicity of the modal approach with the possibility of localization.

In the first method, a fixed set of standard Gaussian RBF centered at a regular grid is used to fit the data by the weighted linear least squares, together with a Tikhonov-type regularization in order to overcome the numerical instability. The advantage of this approach is the possibility to pre-compute and store the necessary data for fast ray tracing or Zernike coefficients recovery.

In the second approach[2], an adaptive and multiscale algorithm is used. Anisotropic Gaussian-type RBF functions are added iteratively to the model for a parsimonious fit of the surface.

Both alternatives have been tested in synthetic and real corneas comparing with the standard Zernike fitting methods. RBF constitute a valuable tool in the reconstruction of the topography of complicated corneas; it might serve as a complementary step to the Zernike basic fit.

[1] A. Martínez-Finkelshtein, A. M. Delgado, G.M. Castro, A. Zarzo and J. L. Alió. *Comparative analysis of some modal reconstruction methods of the shape of the cornea from corneal elevation data*, Investigative Ophthalmology and Visual Science; 50(12) 5639-5645, **2009**.

[2] A. Martínez-Finkelshtein, D. Ramos-López, G.M. Castro and J. L. Alió. *Adaptive cornea modeling from keratometric data*, Investigative Ophthalmology and Visual Science; 52 (8); 4963-4970, **2011**.

P37. APPLICATION OF UHPLC-ORBITRAP-MS FOR THE DEVELOPMENT OF A FAST SCREENING ANALYSIS OF VETERINARY DRUG RESIDUES IN MILK AND POWDERED MILK-BASED FORMULAE

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Screening methods can be applied for rapid identification of veterinary drug (VD) residues in food matrices. Many of these methods are based on the use of liquid chromatography (LC) coupled to mass spectrometry (MS) using low and medium-resolution MS (LRMS/MRMS) analyzers, such as triple quadrupole (QqQ), time of flight (TOF) and quadrupole coupled to TOF (Q-TOF). However, they show certain drawbacks, such as the need for optimizing the acquisition parameters for every single compound in QqQ or the need for extra resolution or sensitivity in TOF or Q-TOF, in complex food matrices. In this sense, the aim of this study is the development of a screening method based on full scan measurements using a high resolution (HRMS) analyzer, Orbitrap, which provides higher resolution than TOF and Q-TOF. Furthermore, the high resolving power of the Orbitrap analyzer permits the determination of the analytes at low concentrations with enhanced accurate mass measurements (< 1 ppm error) required for complex analysis.

This screening method for the determination of 26 VDs in milk and powdered milk-based infant formulae has been developed using an Orbitrap analyzer combined with ultra-high pressure liquid chromatography (UHPLC). The extraction of the analytes was based on the QuEChERS methodology, without sample clean-up. The identification of the target compounds was based on the retention time and the measurement of the accurate mass (error < 3 ppm). Confirmation was carried out applying a pseudo-MS/MS process based on the selection of fragments generated in the HCD collision cell (higher energy collisional dissociation), providing a fragmentation pattern comparable to the typical QqQ spectra. The performance characteristics of the proposed screening method have been evaluated, such as cut-off and unreliability region, linearity and precision. Lower values of cut-off (≤ 5 μg kg^{-1}) as well as the width of the unreliability region (< 4.5 μg kg^{-1}) were obtained, demonstrating the screening capabilities of the Orbitrap analyzer. Furthermore, this analyser was also used for quantification purposes and good performance characteristic were obtained.

This study has demonstrated that the Orbitrap analyzer can be suitable for the determination of VDs in complex food samples by using its screening capabilities. The study of the cut-off and unreliability region also ensures that the proposed method can be used in routine analysis, offering high confidence results.

Acknowledgements. We gratefully acknowledge Spanish Ministry of Science and Innovation (SMSI)-FEDER (AGL 2007-63569 and AGL2010-21370) for financial support. MMAL is grateful to her grant (F.P.U.) from the SMSI (Ref. AP-2008-02811). PPB is grateful for personal funding through the Juan de la Cierva Program (SMSI-European Social Fund, EFS). R.R.G. is also grateful for personal funding through Ramón y Cajal Program (SMSI-ESF)

P38. SIMULTANEOUS DETERMINATION OF PHYTOHORMONES IN VEGETABLES BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY WITH POLARITY SWITCHING

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Phytohormones are produced by plants and therefore they are currently used in ecological agriculture, replacing the massive use of pesticides, fertilizers and synthetic plant growth regulators (analogous to the phytohormones) by natural products.

European Union has been fixed maximum residue limits (MRLs) for some phytohormones; i. e. naphthylacetemide, naphthylacetic acid and 2,4-D (0.050 mg/kg). Therefore, an ultra-performance liquid chromatographic (UHPLC) electrospray ionisation (ESI) tandem quadrupole mass spectrometric method has been developed. Polarity switching has been employed in the determination of six phytohormones in a single run using selected reaction monitoring.

The aim of this study was to achieve a simple extraction method for phytohormones (naphthylacetamide, naphthylacetic acid, gibberellic acid, benzyladenine, indole-3-acetic acid and 2,4-D) to reduce sample handling and increase sample throughput. Therefore QuEChERS procedure was applied; it showed recoveries higher than 75 % and it provided low relative standard deviation (RSD) values.

Separation and determination of the selected phytohormones were carried out by UHPLC coupled to tandem mass spectrometry (UHPLC-MS/MS), using ESI in positive and negative ion modes. The majority of phytohormones included in this study produce negative ions, whereas naphthylacetamide produces a positive ion. Thus, two separate analyses are normally required, one in positive-ion mode and the other in negative-ion mode. Ideally, species of both polarities should be determined in a single analysis via polarity switching. The very fast switching between negative and positive ion mode (20 ms) allowed the suitable detection of naphthylacetamide (ESI+) and benzyladenine (ESI−), two close eluted phytohormones in one single run.

The method was validated and mean recoveries were evaluated at three concentration levels (50, 100, and 250 µg/kg), ranging from 75 to 110 % at the three levels assayed. Intra and interday precision, expressed as RSD, were lower than 20 and 25% respectively. Limits of quantification were equal or lower than 10 µg/kg. The developed procedure was applied to several courgette samples, and naphthylacetic acid, naphthylacetamide and benzyladenine were found in some of the analysed samples.

The QUECHERS and UHPLC-MS/MS methods were developed and validated for the simultaneous determination of gibberellins, cytokinins and auxins in vegetable samples in less than 7 min.

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P39. SYNTHESIS OF DUALLY FUNCTIOLIZED GOLD NANOPARTICLES FOR THE CONSTRUCTION OF SITE-SPECIFIC DELIVERY SYSTEMS

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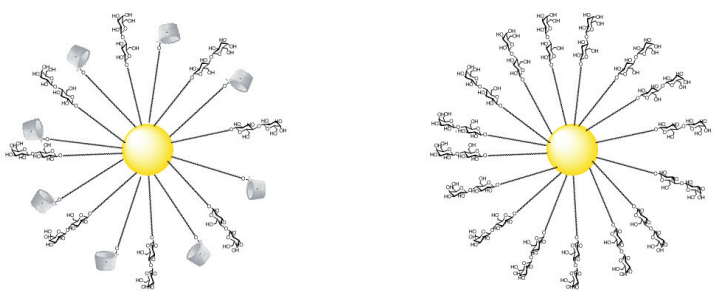
Gold nanoparticles (AuNPs) have recently been a focus of increased interest for their therapeutic potential as drug delivery carriers due to their attractive characteristics such as size, robust stability and biocompatibility.[1,2,3] The goal of gold nanocarriers is to arrive at the diseased tissues after administration into circulatory system and one of the aproaches that is being developed in different systems is the decoration of the carrier surface with targeting ligands for specific recognition by cell surface receptors. AuNPs bearing saccharides may be useful as carriers for transporting drugs to membrane containing specific sugar receptors. In addition, AuNPs provide a versatile scaffold for the construction of multivalent structures of bioactive molecules such as glycosides. This latter feature would allow the use of the so-called glycoside multivalent effect as a means to increase the protein-saccharide binding and therefore could improve the effectiveness of the drug delivery system. As AuNPs can serve as platform for the construction of multifunctional systems, in addition to the targeting ligands, other elements could be appended on the AuNPs surface providing sensing and/or drug encapsulating functions. In this communication, we describe the preparation of AuNPs containing multiple copies of lactose and β-cyclodextrin linked to the NP surface through a polyethylene chain. Lactose is a carbohydrate that is specifically recognized by galectins, a familily of lectins that are overexpressed on cancer cells. In addition, cyclodextrins are able to form host-guest complexes with a large variety of hydrophobic molecules in aqueous solution given the unique nature imparted by their structure. As a result, these molecules have found a number of applications in a wide range of fields such as, pharmaceutical applications for drug release.[4] In order to evaluate the potential of these cyclodextrin-coated glyconanoparticles as site-specific molecular carriers we studied both their affinities for a biological target and the inclusion complexation behaviour with some representative guest molecules by using microcalorimetric titrations. As a biological target the plant lectin from peanut (*Arachis hypogaea*) was chosen. Peanut lectin (PNA) is a homotetrameric protein with a molecular weight of 110 kDa that has one saccharide-binding site per subunit and binds with high affinity β-galactosyl residues through specific binding interactions.[5]



Acknowledgements. We acknowledge the Consejería de Economía, Innovaciónn y Ciencia, Junta de Andalucía and the EU European Regional Development Fund (FQM3141 and in part FQM06903) as well as the EU through a Marie Curie ITN program (CYCLON 237962) for financial support

[1] N. L. Rosi, and C. A. Mirkin, *Chem. Rev.,* **2009**, *105*, 1547

[2] J. Yang, C. H. Lee, H. J. Ko, J. S. Suh, H. G. Yoon, K. Lee, Y. M. Huh and S. Haam, *Angew. Chem., Int. Ed.,* **2007**, *46*, 8836

[3] G. Han, P. Ghosh and V. M. Rotello, *Nanomedicine,* **2007**, *2*, 113

[4] T. Loftsson and D. Duchéne, *International Journal of Pharmaceutics,* **2007**, *329*, 2

[5] H. Lis and N. Sharon, *Chem Rev.,* **1998**, *98*, 637-674

P40. ESTUDIOS ESTRUCTURALES PARA LA DETERMINACIÓN DE LOS MECANISMOS DE FORMACIÓN DE FIBRAS AMILOIDES

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El año 2011 ha sido nombrado año Internacional del Alzheimer, enfermedad neurológica que produce un deterioro progresivo y total de las funciones cognitivas. Es la principal causa de demencia en personas mayores de 65 años y en España están afectadas actualmente unas 600.000 personas, pero se estima que unas 200.000 más están sin diagnosticar. En pocos años estas cifras pueden alcanzar valores equivalentes a los necesarios para que una enfermedad se considere una epidemia.

El desarrollo de la enfermedad de Alzheimer está asociado a la formación de depósitos de proteínas conocidos como amiloides. Estas proteínas proceden de proteínas que se encuentran habitualmente en el cerebro en su correcta conformación tridimensional pero, por causas desconocidas, sufren un proceso por el cual cambian su disposición tridimensional formando agregados fibrilares que son insolubles y resisten la degradación enzimática. Estas fibras en general están formadas por un único tipo de proteína y se caracterizan por una estructura común y repetitiva que adquiere la estructura de hoja plegada beta. Nuestro laboratorio está especializado en la determinación estructural de proteínas, que es la única técnica que puede proporcionar información a nivel molecular de los factores que dan lugar a la estabilización y formación de las fibras amiloides. Actualmente estamos realizando estudios con varias proteínas que nos puede dar una información única para poder determinar los factores moleculares que favorecen los primeros pasos de la formación de los agregados moleculares que conducen a la formación de las fibras amiloides. En concreto, estamos realizando estudios con el dominio SH3 de la Src-tirosina quinasa, proteína implicada en procesos cancerígenos, la cual sufre un proceso conocido como entrecruzamiento de dominios que en los últimos años ha sido reconocido por la comunidad científica como un primer paso del proceso de formación de amiloides. La ventaja de esta proteína es que ha demostrado ser un sistema modelo ideal para realizar estos estudios, ya que nuestro grupo ha descubierto bajo qué condiciones se puede promover la formación de los dominios entrecruzados, pudiendo obtenerlos de forma controlada. Así mismo nuestro grupo ha descrito también la formación de amiloides en otros dominios SH3 homólogos, de los cuales cuenta con información estructural muy valiosa ya que un número considerable de estructuras de estos dominios ha sido obtenida por nuestro grupo a muy alta resolución (cercanas al 1Å) usando radiación sincrotrón. Ello nos ha permitido detectar la presencia de ciertas modificaciones en los aminoácidos presentes en los elementos que conectan la estructura secundaria y que pueden actuar de bisagra para la apertura necesaria para que se origine el entrecruzamiento entre los dominios.

El estudio de los mecanismos por los cuales se da el plegamiento anómalo de las proteínas entrecruzadas, para su posterior evolución a fibras amiloides, es crucial para poder conocer la causa de su funcionamiento anómalo y su implicación en enfermedades tan importantes como la de Alzheimer, Parkinson o Huntington. Las fibras amiloides son también protagonistas en el papel desempeñado por los priones en la conocida como mal de las vacas locas o encefalopatía espongiforme bovina que provoca en humanos la enfermedad de Creutzfeldt-Jakob. Solo conociendo el mecanismo de formación de las fibras amiloides se puede abordar una terapia racional de las enfermedades producidas por las mismas para poder prevenirlas o combatirlas una vez desarrolladas. Debido al progresivo aumento de las expectativas de vida de la población mundial, las enfermedades producidas por la formación de depósitos de amiloides serán no en mucho tiempo las que afecten a un mayor número de seres humanos. Ello requiere que la comunidad científica no se vea afectada de por el *"Alzheimer"* y se ponga en acción para estudiar de forma exhaustiva las causas moleculares de este importante grupo de enfermedades.

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P41. QUANTIFICATION STRATEGIES IN GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF PHENOLIC COMPOUNDS IN WASTEWATER EFFLUENTS

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Phenolic compounds can be found in wastewater (WW) effluents via different sources. For this reason, the development of sensitive analytical methodologies for the simultaneous determination of phenols belonging to different groups is needed to assure the quality of WW effluents. When gas chromatography (GC) is used for the analysis of these compounds, a derivatization step is required in order to improve their chromatographic performance and their sensitivity. A well-known critical point in the analysis of WW is the matrix effect. Consequently, two quantification methods such as matrix-matched calibration (MMC) and standard addition calibration (SAC) have been compared in order to minimize it. The aim of this work was the simultaneous extraction and determination of different phenolic families in WW effluents, evaluating the best quantification strategy for this purpose.

Depending on the treatment received, WW effluents can have different amounts of suspended particulate matter (SPM). In order to verify whether the phenolic compounds were also present in the SPM, a previous distribution study was carried out and it was concluded that the analysis of WW effluents could be limited to the aqueous phase. The target compounds were extracted by solid phase extraction (SPE) without any evaporation step after the elution. A derivatization stage was performed before the chromatographic analysis, and two calibration approaches were compared (SAC and MMC).

The obtained results demonstrated that SAC provides more accurate results than MMC for the quantification of phenols in WW effluents.
The limits of quantification ranged from 0.03 μg L^{-1} to 2.5 μg L^{-1}. Recoveries in the range 60–120%, with precision values ≤ 30% were obtained.

In conclusion, a single extraction method for the simultaneous extraction of different families of phenols in WW effluent samples has been developed using SPE. A distribution study of the compounds between the aqueous phase and the SPM was carried out, verifying that the SPM could be discarded during the extraction. A study using MMC versus SAC was performed in two different treated WW effluents showing that SAC is the most suitable quantification approach. The method was finally applied to real WW samples with satisfactory results.

Acknowledgments. The authors gratefully acknowledge Andalusian Regional Government (Regional Ministry of Innovation, Science, and Enterprise-FEDER) for financial support (Project Ref. P08-RNM-03892). NBB is grateful for her pre-doctoral grant from the aforementioned project. PPB acknowledges for personal funding through Juan de la Cierva Program (Spanish Ministry of Science and Innovation-European Social Fund (SMSI-ESF)). RRG is also grateful for personal funding through Ramón y Cajal Program (SMSI-ESF).

P42. DETERMINATION OF CHLOROTHALONIL IN DIFFICULT-TO-ANALYSE VEGETABLE MATRICES USING VARIOUS MULTIRESIDUE METHODS

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The molecular characteristics of chlorothalonil can cause particular determination difficulties in some vegetable commodities such as leek or garlic. These difficulties are mainly related to the low recoveries obtained using common multi-residue methods (MRMs) - a consequence of the very high interaction level with natural components in the matrix. These shortcomings were pointed out in the last European Proficiency Test for pesticide residues on Fruits and Vegetables, where false negatives for chlorothalonil in leek were observed at around 50%.

In this study we have evaluated the Ethyl Acetate, the Dutch mini-Luke and the QuEChERs MRMs to compare their capabilities for chlorothalonil determination using GC-MS/MS in both the electron impact ionization (EI) and negative chemical ionization (NCI) modes. Best recoveries (in the range of 100-120%, with an RSD below 20%) were obtained using the Dutch mini-Luke method. Lower values (52-70%) were obtained for Ethyl Acetate whereas no recovery was obtained when the QuEChERs method was applied. Furthermore, tomato matrix was also included in the experiments in order to facilitate the comparability of results.

Two ionization modes, electron impact ionization (EI) and negative chemical ionization (NCI) in GC-MS/MS were applied to evaluate their respective advantages and disadvantages for quantification and identification. As expected, NCI showed limits of detection (LODs) 5 to 10 times lower than EI. However, in both cases, the LODs were still below 10 μg Kg^{-1}.The proposed optimal method was applied for chlorothalonil determination in leek and garlic with good results - in accordance with the European Union (EU) Analytical Quality Control (AQC) Guidelines for pesticides analysis.

P43. DESIMETRIZACIÓN DE *P,P*-DIFENILAMINOFOSFACENOS MEDIANTE PROCESOS DE *ORTO*-LITIACIÓN-ADICIÓN-ELECTROFÍLICA

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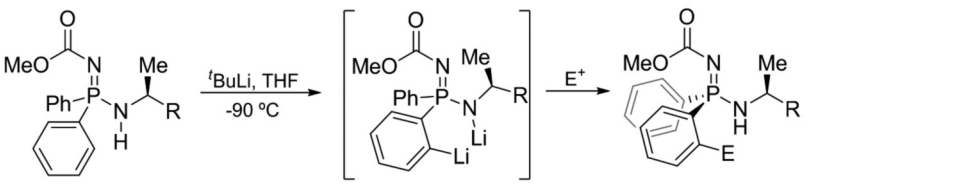
La litiación dirigida a la posición *orto* ha superado a la sustitución electrofílica aromática clásica como principal metodología para introducir distinto grado y tipo de funcionalización en anillos aromáticos de manera regioselectiva. Los grupos directores ejercen la doble función de aumentar la acidez de los protones orto y aproximar la base a dicha posición. Existe una amplia variedad de grupos fosforados que se han utilizado para dirigir la litiación hacia la posición *orto* de un anillo *P*-fenílico, aunque el estudio de funciones fosforadas que contienen hidrógenos ácidos se ha iniciado recientemente.[1] Los aminofosfacenos son compuestos que contienen la agrupación N-P=N, que les hace susceptibles de participar en procesos de metalación dirigida y les confiere una gran capacidad de complejación a metales.

El trabajo que aquí se presenta consiste en el uso de la función aminofosfaceno como director de metalación *orto*, y el posterior tratamiento con electrófilos de muy diversa naturaleza con el propósito de formar nuevos enlaces carbono-carbono y carbono-heteroátomo. El procedimiento permite acceder a una gran variedad de productos funcionalizados en posición orto con sustituyentes tales como Me, I, R^1R^2COH, CH_2CHCH_2, $SnMe_3$, $SiMe_3$, y Ph_2PO con elevados rendimientos, algunos de los cuales presentan una disposición de heteroátomos adecuada para actuar como ligandos bidentados en química de coordinación. Utilizando sustratos quirales en el grupo amino se obtienen productos enantioméricamente puros desimetrizados en el grupo PPh_2 con excelente diastereoselectividad.



1 R = Me
(*S*)-**2** R = Ph

E = Me, CH_2CHCH_2, $C(OH)(Ph)_2$, $CH(OH)(Ph)$, I, $SnMe_3$, $SiMe_3$, Ph_2PO.

[1] (a) Fernández, I.; Oña-Burgos, P.; Ruiz-Gómez, G.; Bled, C.; García-Granda, S.; López-Ortiz, F. *Synlett* **2007**, 611. (b) Yáñez, V.; Iglesias, M. J.; López-Ortiz, F. *JOC*, enviado.

P44. ^{7}Li,^{15}N{^{1}H} HMQC NMR: CONNECTING NUCLEI AT NATURAL ABUNDANCE!

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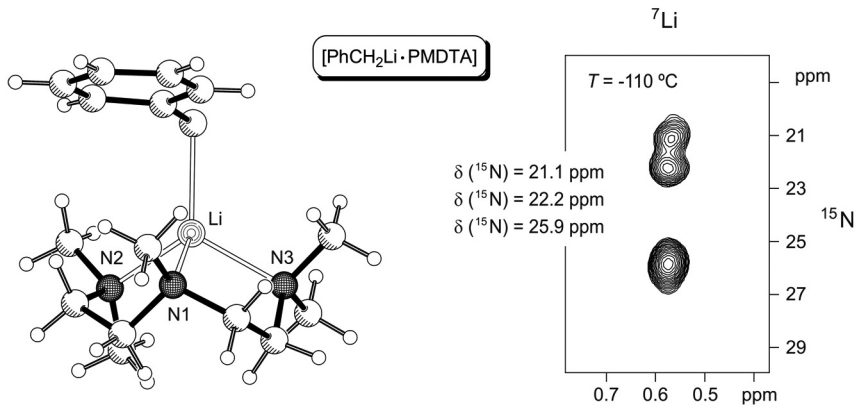
A straightforward natural abundance 2D HMQC NMR experiment between *lithium-7* and *nitrogen-15* is described. This fast, reliable and routinely implementable method represents a powerful spectroscopic tool to study species in solution containing nitrogen-lithium bonds, where for instance isotopic labeling is not available or only possible with synthetic difficulties.[1] We report herein the use of this methodology together with nOe, PGSE diffusion NMR, and X-ray crystallography to unravel the structure of some old and new lithiated species, where PMDTA, TMEDA and (-)-sparteine play a significant role.



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[1] Fernández, I., Oña Burgos, P., Armbruster, F., Krummenacher, I., Breher, F. *Chem. Commun*., 2586-2588 (2009).

P45. PREPARACIÓN Y USO DE SISTEMAS DE LIBERACIÓN CONTROLADA DE NITRATOS PARA PREVENIR LA CONTAMINACIÓN MEDIOAMBIENTAL

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Los sistemas de liberación controlada están diseñados para suministrar niveles óptimos de ingrediente activo (plaguicida y/o fertilizante) en el lugar preciso y en un periodo de tiempo deseado, reduciendo las pérdidas por lixiviación y evaporación, con lo que disminuyen la necesidad de aplicarlos frecuentemente y en mayor cantidad de la realmente requerida. Un incremento en los niveles de nitratos pone en peligro la aptitud del agua para su uso potable, provoca su eutrofización causando un gran daño a ríos, lagos, humedales y aguas costeras. Las consecuencias son pérdidas de carácter económico y alto riesgo para la salud. Por otra parte, los nitratos presentes en la solución del suelo pueden ser reducidos por cierto tipo de bacterias hacia las formas gaseosas de N_2, NO y N_2O que pasan a la atmósfera del suelo y de ésta a la atmósfera exterior. El papel de N_2O es incuestionable por su directa implicación en el fenómeno de contaminación atmosférica conocido como efecto invernadero.

Teniendo en cuenta todo lo indicado, el objetivo de este trabajo es la preparación y ensayo de sistemas de liberación controlada de nitratos como alternativa a las formulaciones existentes, ya sean las clásicas o las escasamente utilizadas formulaciones de liberación lenta[1,2]. Se han empleado para ello matrices poliméricas o sólidos inorgánicos como medios encapsulantes, e investigado los agentes coadyuvantes de características más apropiadas para conseguir una encapsulación más efectiva, con la finalidad de prevenir la contaminación del medio hídrico por nitratos. Pretendemos así, contribuir por un lado, a preservar este importante recurso natural, imprescindible para una producción agrícola sostenible, y por otro, a evitar los efectos tóxicos de las aguas contaminadas, cuando éstas se usan para consumo humano y animal.

Los resultados obtenidos podrán ser utilizados para diseñar nuevas estrategias de prácticas agrícolas con objeto de hacer frente a la grave contaminación del medio acuático.

Agradecimientos. Los autores de este trabajo agradecen a la Agencia Española de Cooperación Internacional y Desarrollo (AECID) la financiación concedida a través del proyecto (A/017167/08) mediante el que se ha realizado esta investigación.

[1] María Dolores Ureña-Amate, Naoufal Debbagh Boutarbouch, María del Mar Socias-Viciana, Emilio González-Pradas, Applied Clay Science 2011, 52, 368-373.

[2] Socías-Viciana, M.M., Ureña-Amate, M.D., González-Pradas, E., García-Cortéz, M.J., López-Teruel, C., 2008. Clays Clay Miner. 56 (1), 2-9.

P46. DATABASES AND COMPREHENSIVE ANALYSIS OF PESTICIDES AND VETERINARY DRUGS USING LIQUID CHROMATOGRAPHY-ORBITRAP HIGH RESOLUTION MASS SPECTROMETRY

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The current legislation in food implies the determination of a high number of residues and organic contaminants, such as veterinary drugs (VDs) and pesticides. Low quantification limits, wide scope analysis and efficiency are demanded by food laboratories. Some of these requirements are hardly complied with current analytical techniques, which are based on chromatographic techniques coupled to low resolution mass spectrometry (LRMS) analyzers, such as triple quadrupole (QqQ). However, LRMS shows limitations when a huge number of contaminants must be simultaneously analyzed. Besides, QqQ are only focused on target analysis, discarding relevant information about other non-target contaminants and residues. These drawbacks can be overcome by the use of high resolution mass spectrometry (HRMS) instruments, such as Orbitrap, which operates in the full scan mode (theoretically, no limitations in number of monitored compounds) and providing accurate mass measurements and the possibility of performing retrospective analysis for the determination of non-target compounds.

A group of ~400 pesticides and VDs were included in the database. The average MS parameters were established for the analysis of the compounds by UHPLC-Orbitrap-MS. The characteristic ion of each analyte was defined for identification purposes, ($[M+H]^+$, $[M+Na]^+$, $[M-H]^-$, $[M+CH3COO]^-$, etc.). Chromatographic parameters involved in the separation of the target compounds were also optimized. Finally, the selected compounds were characterized by retention time, molecular mass (mass accuracy) and spectrum.

A screening method has been developed for the determination of ~400 compounds by UHPLC-Orbitrap-MS, establishing a database that includes retention time and characteristic ions of the target compounds. The proposed database allows the automated search of the analytes, and then, the confirmation and quantification of the detected compounds can be carried out within the same injection.

The full scan mode permits the acquisition of all the ions produced in the source and, thus, the number of analytes is (theoretically) unlimited in the selected *m/z* range. On the other hand, the HRMS instruments can improve the identification/confirmation process with the information provided by accurate mass measurements.

Acknowledgements. We gratefully acknowledge Spanish Ministry of Science and Innovation (SMSI)-FEDER (AGL2010-21370) for financial support. MLGP acknowledges her grant (F.P.I.) from the Spanish Ministry of Science and Innovation (Ref. AGL2010-21370). RRG is also grateful for personal funding through the Ramón y Cajal Program (SMSI-European Social Fund, ESF). PPB is grateful for personal funding through the Juan de la Cierva Program (SMSI-ESF).

P47. USE OF AN ACCURATE-MASS DATABASE FOR THE SYSTEMATIC DENTIFICATION OF TRANSFORMATION PRODUCTS OF ORGANIC CONTAMINANTS IN WASTEWATER EFFLUENTS

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In the last years, a large number of compounds have been pointed out as possible environmental contaminants. These compounds usually named as emerging contaminants belong to different chemical groups and come from different uses. Pharmaceuticals, personal care products, flame retardants, plasticizers, etc., have been and are currently investigated in natural and wastewater to gain knowledge about their presence, behaviour and fate in the environment and, more importantly, about its possible impact on environmental quality. Current research efforts in environmental analytical chemistry include the development of analytical methodologies with the ability of screening and detect hundreds of contaminants at ultra-trace levels. This performance should not be limited to known species (targeted analysis) but also to unknown or unexpected species (untargeted approaches) for which no standards are available in advance to the analysis. In this study, a systematic approach is proposed to assist and simplify the identification of transformation products (TPs) of organic contaminants which might be even more hazardous and persistent than the parent compounds. It is based on the use of characteristic fragmentation undergone by organic contaminants during MS/MS fragmentation events, and the relationship and consistency with the transformations experimented by these chemicals in the environment or during water treatment processes. Therefore, the fragmentation pathways of the parent species can be used to predict possible TPs since bonds that are easily cleaved under collision-induced dissociation (CID) conditions are likely to be cleaved in reactions at real ambient conditions. With this in mind, a database containing accurate-mass information of 147 compounds and their main fragments generated by CID MS/MS fragmentation experiments was created using an LC-QTOF-MS/MS system. The developed database was applied to the identification of tentative TPs and related unexpected compounds in eight wastewater effluent samples. The approach comprises basically three stages: (a) automatic screening, (b) identification of possible TPs and (c) confirmation by MS/MS analysis. Parameters related to the search of compounds in the database have been optimized and their dependence with the exhaustiveness of the study evaluated. Eight degradation products were identified with a high grade of accuracy and three of them were confirmed by analysis of the corresponding analytical standards.

Acknowledgements: The authors acknowledge the Spanish Ministry of Science and Innovation-Fondos FEDER (Project CTQ2010-20740-C03-03) and the Spanish Ministry of Education and Science (Program Consolider Ingenio 2010 Ref. CSD2006-00044 (TRAGUA)) for economical support.

P48. ADDITION OF KETENE SILYL ACETALS TO EPOXIDES CATALYZED BY COPPER COMPLEXES

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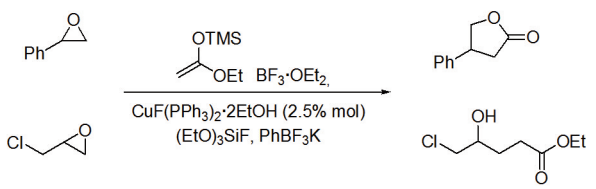
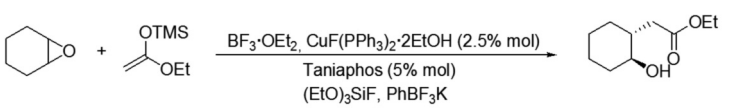
The allylation of epoxides is a common process used in the preparation of interesting natural products that can be achieved with strong nucleofiles like allymagnesium or lithium derivatives. However, it can also be done under non basic conditions using moisture-, oxygen-, and thermally-stable allylsilanes or allystannanes as nucleophiles.[1] However, due to the high toxicity of tin derivatives, the environmentally friendly organic silicon derivatives seem to be a better choice as starting materials. While the enantioselective desymmetrization of achiral meso epoxides is an interesting way to create new stereogenic centers, it is already known that in the absence of the Lewis acid, the nucleophilic opening of such unreactive epoxides is a very slow reaction. Activation by coordination of a Lewis acid, such as BF_3 OEt_2, to the oxygen atom of the oxirane is required. Indeed, several methodologies using asymmetric catalytic systems have been successfully developed for the enantioselective opening of meso-oxiranes by noncarbon nucleophiles (including the CN group),[2] as well as organometallic species, mainly organolithium reagents. We present here for the first time the desymmetrization of meso epoxides by ketene trimethylsilyl acetals catalyzed by a copper(I) fluoride-phosphine complex. The attempts of addition of allysilanes, allylsiloxanes and ketene silyl acetals to epoxides in the presence of copper catalysts were unsuccessful due to the low reactivity of epoxides towards these mild reagents, even in the case of the very reactive styrene oxide. However, when the reaction was performed in the presence of BF_3 OEt_2, a clean reaction took place, affording the corresponding addition products. It has been proposed that a copper enolate generated through transmetalation is the actual nucleophile, and the addition of a stoichiometric amount of $(EtO)_3SiF$ to facilitate the rate-determining catalyst turnover is essential.[3]



These results, clearly indicating that copper enolates are compatible with boron trifluoride activation of oxiranes, prompted us to check if the reaction could be applied to the desymmetrization of meso epoxides. Thus, we treated cyclohexene oxide with a ketene trimethylsilyl acetal in the presence of both BF_3 OEt_2 and a chiral copper(I) complex generated *in situ*. We were delighted to observe a clean addition reaction. The degree of stereocontrol of this reaction is being currently studied.



Acknowledgements. To Ministerio de Ciencia e Innovacion and Junta de Andalucia for financial support (Projects CTQ2010-17115 and Proyecto de Excelencia P08-CVI-03591) and to MICIN for a scholarship to C. Hernández-Cervantes

[1] E. Vrancken, A. Alexakis, P. Mangeney *Eur. J. Org. Chem.* **2005**, 1354–1366

[2] M. C. Willis, *J. Chem. Soc., Perkin Trans. 1* **1999**, 1765–1784.

[3] K. Oisaki, D. Zhao, M. Kanai, M. Shibasaki *J. Am. Chem. Soc.* **2006**, *128*, 7164-7165

P49. DIHYDROBENZO[*b*]FURAN SYNTHESIS CATALYZED BY IRIDIUM-PORPHYRIN COMPLEXES

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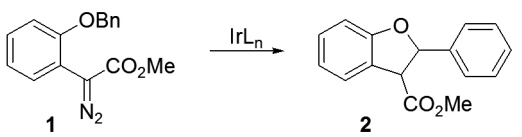
Functionalization of C–H bonds by metal carbenoid insertion is a process that usually shows high regio- and stereoselectivity. Its most useful application is the intramolecular reaction that occurs with high selectivity for the formation of five-membered ring products. The use of dimeric Rh(II) complexes in this area has been extensively developed.[1] The mechanism of this reaction involves simultaneous C–H bond breaking, C–C and C–H bonds formation, and the dissociation of the metal catalyst from the original carbene center.

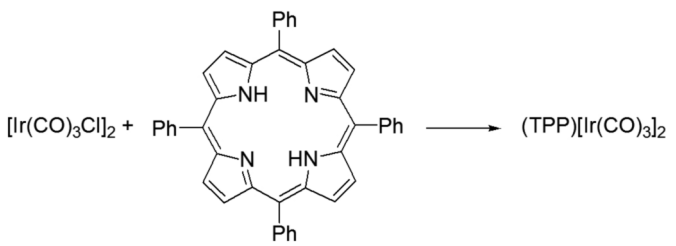
Recent studies show that iridium complexes are also capable of catalyzing C–C bond coupling via carbenoid insertion into C–H bonds. Katsuki et al. reported that iridium(III)–salen complexes catalyze asymmetric intermolecular C–H insertion of carbenes at the α position of THF and at the allylic position of 1,4-cyclohexadiene.[2] In both cases, the reactions are highly enantioselective and in the case of THF, highly diastereoselective. Simple α-methyl-α-diazoacetate gave the insertion without the usual β-H-elimination competing reaction.

We have explored the intramolecular C-H insertion to form dihydrobenzofurans (1→2) promoted by iridium complexes.



1 **2**

A selection of Ir(0), Ir(I) and Ir(III) catalysts were assayed. In the presence of Ir(0) only degradation products were obtained. Ir(III) gave mainly dimerization, together with the desired cyclization products in a small ratio. Excellent results were obtained with the binuclear iridium (I) porphyrin complex (TPP)[$Ir(CO)_3$]$_2$ described by Kadish,[3] which promoted fast cyclization at -78ºC in yields higher than 98%.



Acknowledgements. To Ministerio de Ciencia e Innovacion and Junta de Andalucia for financial support (Projects CTQ2010-17115 and Proyecto de Excelencia P08-CVI-03591) and to MICIN for a scholarship to C. López-Sánchez).

[1] Davies H.M.L.; Manning J.R. *Nature* **2008**, *451*, 417–424.

[2] Suematsu, H.; Katsuki, T. *J.Am.Chem.Soc.* **2009**, *131*, 14218-14219.

[3] Kadish, K.M.; Deng, Y.J.; Yao, C.L.; Anderson, J.E. *Organometallics* **1988**, *7*, 1979-1983.

P50. BENEFITS AND PITFALLS OF THE APPLICATION OF SCREENING METHODS FOR THE ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

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Most of the analytical methods currently applied in food control laboratories, both administration and private, are aimed at the identification of target compounds. These methods employ highly selective analytical techniques, which mean that compounds not included in these methods escape detection. Given that the current need to detect the compounds unauthorized use is prohibited, whose presence is not expected in the samples, it is necessary to extend the scope of the methods to include a large number of potential compounds. Thus, an alternative can be the use of quick and simple screening methods to allow positive identification, within a wide range of compounds, along with subsequent confirmation and quantification of them.

The goal of this work has been gaining an insight into the performance of a based accurate mass measurement screening for a wide range of pesticide residues in different matrices using LC-QTOF-MS and library-based automated detection. With this aim, 97 pesticides at three concentration levels (20, 50 and 100 μg L^{-1}) were investigated in representative fruit and vegetable matrices belonging to two different commodities groups: high water content (tomato, pepper, zucchini and leek) and high acid and water content (orange)[1]; and we investigated how sensitivity, matrix interference effects, calibrant suppression, efficiency of algorithm search, etc. affects in the automatic identification.

Sensitivity had been point as the main pitfall of the automatic search. Also, some failures in the software for automatic data treatment in terms of analysis of isobaric compounds, isotopic information, spectra deconvolution and speed data processing have been detected, and they should be improved.

Acknowledgements. Regional Government of Andalusia (Project ref. AGR-4047); European Commission, DG SANCO (SANCO/2005/FOOD SAFETY/0025-Pesticides in Fruit and Vegetable). A. L. acknowledges the FPU fellowship (Research Teacher Training) from Spanish Ministry of the Science and Education.

[1] Method validation and quality control procedures for pesticide residues analysis in food and feed. Document No. SANCO/10684/2009.

P51. PRODUCCIÓN DE ß-AMINOÁCIDOS A TRAVÉS DE UN SISTEMA BIOENZIMÁTICO

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Los ß -aminoácidos presentan propiedades farmacológicas únicas, ya sea en su forma libre o como componentes de una gran variedad de productos naturales[1]. Esto ha propiciado que numerosos grupos centren su investigación en la síntesis de ß-aminoácidos no naturales para el diseño de nuevos fármacos[2-3] y como precursores de ß-péptidos ya que presentan importantes ventajas y propiedades farmacológicas frente a los formados por a-aminoácidos[4-5].

Los ß-aminoácidos naturales ß-alanina y a-metil-ß-alanina son producidos en la naturaleza mediante la degradación reductiva de pirimidinas, a través de una cascada enzimática llevada a cabo por las enzimas dihidropirimidina deshidrogenasa (EC 1.3.1.2), dihidropirimidinasa (EC 3.5.2.2) y ß-ureidopropionasa (EC 3.5.1.6)[6]. En nuestro grupo se ha llevado a cabo un amplio estudio de la dihidropirimidinasa de *Sinorhizobium meliloti* [7] y la ß-ureidopropionasa de Agrobacterium tumefaciens C58[8], demostrando la alta promiscuidad de sustrato de ambas enzimas. Tras estos resultados se ha considerado la posibilidad de producir ß-aminoácidos a partir de dihidrouracilos empleando un sistema formado por ambas enzimas recombinantes, estrategia no considerada hasta la fecha. Resultados preliminares muestran la posible síntesis de ß-alanina, a-metil-ß-alanina, ß-homoalanina y a-fenil-ß-alanina mediante este sistema.

Agradecimientos. Este trabajo ha sido financiado por el Ministerio de Ciencia e Innovación, el Fondo Social Europeo (FSE), el Fondo Europeo para el Desarrollo Regional (FEDER) y la Consejería de Economía, Innovación y Ciencia, a través de los proyectos CVI-02651, P09-TEP-4691 y BIO2011-27842 y una beca predoctoral BES-2008-003751.

[1] E. Juaristi and V. A. Soloshonok. Wiley-Interscience **2005**, 2ª ed., Hoboken, NJ, USA.

[2] L. Zimmerli, B. H. Hou, C. H. Tsai, G. Jakab, B. Mauch-Mani and S. Somerville. Plant J. **2008**, 53, 144-156.

[3] F. Palacios, C. Alonso and J. M. de los Santos. Chem. Rev. **2005**, 105, 899-931.

[4] D. Seebach, A. K. Beck and D. J. Bierbaum. Chem. Biodiversity **2004**, 1, 1111-1239.

[5] S. Reinelt, M. Marti, S. Dédier, T. Reitinger, G. Folkers, J. A. López de Castro y D. Rognani. J. Biol. Chem **2001**, 276, 24525-24530.

[6] C. Wasternack. Pharmacol. Ther. **1980**, 8:629–651.

[7] S. Martinez-Rodriguez, A. I. Martinez-Gomez, J. M. Clemente-Jimenez, F. Rodriguez-Vico, J. M. Garcia-Ruiz, F. J. Las Heras-Vazquez, and J. A. Gavira. J. Structural Biology **2010**, 169(2):200–208.

[8] A. I. Martinez-Gomez, S. Martinez-Rodriguez, J. Pozo-Dengra, D. Tessaro, S. Servi, J. M. Clemente-Jimenez and F. Rodriguez-Vico, F. J. Las Heras-Vazquez. Appl. Environ. Microbiol. **2009**, 75:514-520.

P52. CARBAMOYLASES: BIOTECHNOLOGICAL APPLICATION IN KINETIC RESOLUTION OF *N*-SUBSTITUTED AMINO ACIDS

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Enzymatic kinetic resolution is a widely used biotechnological tool for the production of enantiomerically pure/enriched compounds. This technique takes advantage of the enantioselectivity or enantiospecificity of an enzyme for one of the enantiomers of a racemic substrate to isolate the desired isomer. *N*-Carbamoyl-D- and L-amino acid amidohydrolases (D- and L-carbamoylases) are model enzymes for this procedure due to their strict enantiospecificity[1]. This enzyme has become crucial in the industrially used multienzymatic system known as "Hydantoinase Process" for the production of optically pure D-amino acids, where the kinetic resolution accomplished by coupling an enantioselective hydantoinase and the enantiospecific carbamoylase is enhanced by the enzymatic/chemical dynamic kinetic resolution of the low-rate hydrolyzed substrate[2]. During the last years, we have focused our research on finding different L-carbamoylases with different substrate specificities to enhance the possibilities of the Hydantoinase Process for the production of non-natural optically pure L-amino acids[3-7], as the enantioselectivity of D-hydantoinases hampered the profitability of this process[8]. Through different approaches (Fig. 1), we have been able to produce several optically pure amino acids (e.e. ≥ 99 %) starting from different *N*-substituted amino acids or 5-mono-substituted hydantoins (Soriano-Maldonado and Rodríguez-Alonso et al., works in preparation). Among them, L-homophenylalanine, precursor of enalapril, was proved to be generated *in situ* without further purification processes.

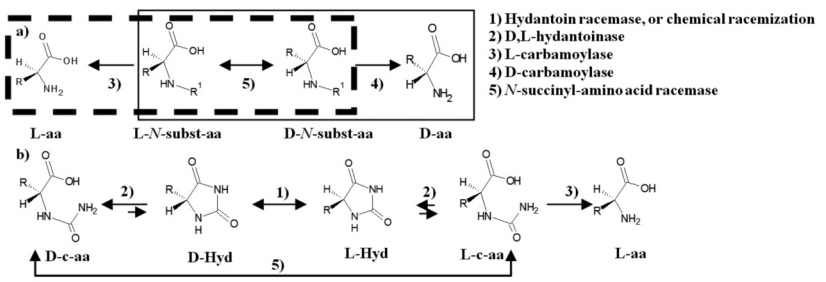

Figure 1. Different applications of D- and L-carbamoylases. a) "Carbamoylase process" (R^1=carbamoyl; L-system, dashed line; D-system, full line). b) "Modified hydantoinase process" (L-system). Subst-aa: *N*-substituted-amino acid (*N*-formyl-, *N*-carbamoyl- or *N*-acetyl-amino acids); hyd: 5-monosubstitued hydantoin; c-aa: *N*-carbamoyl-amino acid, aa: amino acid.

[1] S. Martínez-Rodríguez, A.I. Martínez-Gómez, F. Rodríguez-Vico, J.M. Clemente-Jiménez, F.J. Las Heras-Vázquez. *Appl. Microbiol. Biotechnol.* **2010**, 85, 441-458.

[2] J.M. Clemente-Jiménez, S. Martínez-Rodríguez, F. Rodríguez-Vico, F.J. Las Heras-Vázquez. *Recent Pat. Biotechnol.* **2008**, 2, 35-46.

[3] S. Martínez-Rodríguez, J.M. Clemente-Jiménez, F. Rodríguez-Vico, F.J. Las Heras-Vázquez. *Mol. Microbiol. Biotechnol.* **2005**, 9, 16-25.

[4] S. Martínez-Rodríguez, A. García-Pino, F.J. Las Heras-Vázquez, J.M. Clemente-Jiménez, F. Rodríguez-Vico, R. Loris, J.M. García-Ruiz, J.A. Gavira. *Acta Crystallogr. F.* **2009**, 64, 1135-1138.

[5] A.I. Martínez-Gómez, S. Martínez-Rodríguez, J. Pozo-Dengra, D. Tessaro, S. Servi, J.M. Clemente-Jiménez, F. Rodríguez-Vico, F.J. Las Heras-Vázquez. *Appl. Environ. Microbiol.* **2009**, 75, 514-520.

[6] J. Pozo-Dengra, A.I. Martínez-Gómez, S. Martínez-Rodríguez, J.M. Clemente-Jiménez, F. Rodríguez-Vico, F.J. Las Heras-Vázquez. *Biotechnol. Progr.* **2010**, 26, 954-959.

[7] A.I. Martínez-Gómez, M. Andújar-Sánchez, J.M. Clemente-Jiménez, J.L. Neira, F. Rodríguez-Vico, S. Martínez-Rodríguez, F.J. Las Heras-Vázquez. *J. Chrom. B.* **In press**, doi:10.1016/j.jchromb.2011.04.008.

[8] B. Wilms, A. Wiese, C. Syldatk, R. Mattes, J. Altenbuchner. *J. Biotechnol.* **2001**, 9, 19-30.

P53. PAMAM-BASED ELECTROACTIVE GLYCODENDRIMERS FOR SENSING LECTINS

M. C. Martos-Maldonado, J. M. Casas-Solvas, I. Quesada-Soriano, L. García Fuentes and A. Vargas-Berenguel

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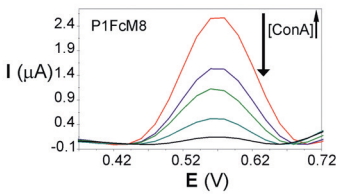
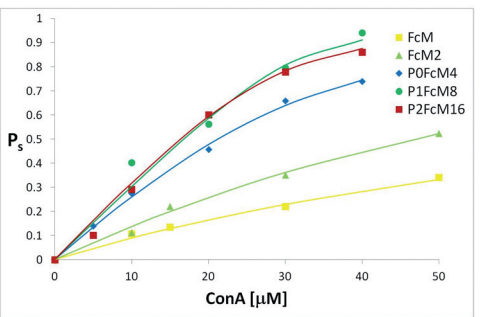
Important biological events such as viral and bacterial infections, cell-cell adhesion, inflammatory and immune response, fertilization, and cancer metastasis are governed by multivalent interactions between carbohydrates and cell-surface proteins.[1] Inherent in the advance of glycomics, is the challenge to develop synthetic tools that can be used to inhibit, modulate, detect and probe those interactions.[2] In this respect, ferrocene-containing carbohydrates could be of particular interest as the reversible and tunable redox properties of the ferrocene moiety could be applied in the development of molecular devices for the detection of carbohydrate-protein interactions, as well as in a redox switchable control of such interactions.

Considering the above, we have synthesized electroactive PAMAM-based glycodendrimers which include 4 (**P0FcM4**), 8 (**P0FcM8**) or 16 (**P0FcM16**) ferrocene-mannose units. The capabilities of these compounds to recognize Concanavalin A (Con A) and to act as electrochemical sensors for such lectin has been studied using calorimetric and electrochemical methods and compared with results obtained for ferrocene-mannose conjugates synthesized previously that contain one (**FcM**) or two (**FcM2**) mannose units.[3]





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[1] (a) Dwek, R. A. *Chem Rev.* **1996**, *96*, 683-720. (b) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637-674.

[2] Timmer, M. S.; Stocker, B. L.; Seeberger, P. H. *Curr. Opin. Chem.Biol.*, **2007**, *11*, 59–65.

[3] Casas-Solvas, J. M.; Ortiz-Salmeron, E.; Garcıa-Fuentes, L.; Vargas-Berenguel, A. *Org. Biom. Chem.* **2008**, *6*, 4230-4235.

P54. PURIFICATION OF DHA-ETHYL ESTER FROM BY-PRODUCTS OBTAINED FROM DICENTRARCHUS LABRAX AND SPARUS AURATA

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Docosahexaenoic acid (DHA, 22:6*n*-3) is a *n*-3 polyunsaturated fatty acid (*n*-3 PUFA) which has been widely reported to possess a beneficial physiological activity decreasing the risk of suffering cardiovascular disorders. Also, it is essential for a suitable visual and cognitive development in childhood.

DHA is mainly found in marine oils, being fish fillet the most common dietary supply. However, this option is not environmentally sustainable at a long term due to the reduced global fish stocks. This way, new DHA sources must be found to meet the increasing demand of this FA. In this study, viscera (gonads, intestine and stomach) from *Dicentrarchus labrax* and *Sparus aurata*, which are considered as by-products in the fishing industry, have been used to obtain a purified DHA extract.

Oil was extracted from viscera using *n*-hexane as solvent. After that, it was derivatized to obtain the corresponding FA ethyl esters (FAEEs). DHA-EE extract was purified by means of a gravimetric normal-phase chromatographic column with silica gel and silver nitrate as stationary phase. The mobile phase was composed by volumetric mixtures of *n*-hexane, acetone and ethanol with increasing polarity.

As a result of this work, a purified DHA extract (100% DHA purity on total FAs) has been obtained. Furthermore, all solvents employed in this methodology are currently allowed for food industry processing.

P55. NUTRIENT COMPOSITION OF BY-PRODUCT FROM FISH PROCESSING PLANTS

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Fish processing and marketing as fillets adds value to the product from direct marketing; however, the edible part does not exceed 40% of fresh weight of the animal. The by-products of the fishery and aquaculture industry include heads, fins, skin, black meat and viscera (gonads, intestine and stomach), being the latter characterized by a high moisture content, high biological value protein and oils with high content in essential fatty acids (FAs) that are required by many industries including food, agriculture, aquaculture and pharmaceuticals.

This work aims to analyze chemical composition of byproduct obtained in a processing plant of cephalopods, octopus (*Octopus vulgaris*) and cuttlefish (*Sepia apama*) and the viscera obtained in processing plant of cultured sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) to assess cost-effective use of this by products.

Cuttlefish by-products showed high moisture content; 20% dry matter with a high protein percentage (70%) and a very low lipid content (less than 3%). These features make this an ideal material for use as high-quality protein source. Octopus viscera had 40% dry matter, for a 55% protein. In this species, lipids were higher than in cuttlefish by-products (under 20%), being the fatty acid profile rich in polyunsaturated FA content, with noticeable amounts of EPA and DHA.

Viscera obtained from cultured sea bream and sea bass had higher content of dry matter (50%) than the same from cuttlefish and octopus. In this case, protein content was the lowest (30%), but for a high lipid value (under 55%). In all cases, FA profile had an optimal composition for the use of these products as raw material to attempt the concentration of polyunsaturated FAs, which significantly increase the added value of processed by-products of aquaculture, as ingredients highly valued for the food industry.

P56. PURIFICATION PROCESSES OF 2-MONOACYLGLYCEROLS OBTAINED FROM HYDROLYSIS OF ECHIUM PLANTAGINEUM SEED OIL

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Triacylglycerols (TAGs) containing specific fatty acids in designated positions are called structured lipids (SLs), and they have become of interest because beneficial effects of these TAGs are closely associated to their structures. Mammalian pancreatic lipase hydrolyzes the ester linkages at the *sn*-1 and *sn*-3 positions with a preference for medium-chain over long-chain fatty acids. This way, MLM-type SLs containing medium-chain fatty acids (M) at *sn*-1 and *sn*-3 positions and long-chain fatty acids (L) at the *sn*-2 position could provide an improved absorption of the resulting *sn*-2 monoacylglycerol (*sn*-2 MAG) through the intestinal mucosa.

Sn-2 MAGs are convenient reagents for the synthesis of structured lipids. However, the yields in the synthesis and purification methodologies depend on the impact of acyl migration which isomerizes the *sn*-2 MAGs to *sn*-1 MAGs. This way, that factor should be taken into account when obtaining and purifying the desired *sn*-2 MAGs.

The objective of this study was the obtaining of *sn*-2 MAGs from Echium plantagineum seed oil containing gamma-linolenic acid (GLA, 18:3*n*-6) and stearidonic acid (SDA, 18:4*n*-3) as the most relevant components. Synthesis yield and acyl migration rate caused during purification processes have been reported.

Echium plantagineum seed oil was hydrolyzed and the resulting *sn*-2 MAGs were purified by chromatographic column and by liquid-liquid extraction. Recovery yield and acyl migration rate (via ^{1}H-NMR analysis) were determined.

High acyl migration rates have been obtained when both purification methods are employed. This way, *sn*-1 MAGs are 1.5 times more abundant than *sn*-2 MAGs after the purification processes have been carried out. Chromatographic column purification offer better results for MAG purification after hydrolysis (more than 95%) than liquid-liquid separation (up to 50%).

P57. GONAD COMPOSITION OF FARMED AND WILD SEA URCHINS PARACENTROTUS LIVIDUS

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The potential marketing of the edible sea urchin (*Paracentrotus lividus*) has considerably increased in the last two decades due to both good aroma and taste. The edible portion comprises the gonads, which are half-moon-shaped, yellow to orange, and constitute approximately 10% on total weight. The global exploitation of natural resources of the sea urchin fishery has prompted increased interest in aquaculture of sea urchins (*echiniculture*). Successful commercial farming of sea urchin depends of several factors related to the consumer acceptance, mainly an adequate quality of the farmed specimens compared with the wild ones. One of the main quality indexes in farmed fish for human consumption is the fatty acid (FA) profile of lipid stored in gonads. FA composition in this species, as commonly occurs in marine organisms, must be considered as the result of endogenous synthesis and exogenous dietary supply. Also, differences in total fat amount between male and female gonads may be due to the different reproductive effort, which is higher in females.

The objective of this study was to investigate the FA composition of the gonad in male and female sea urchins, and to compare farmed and wild species obtained from the south coast of Spain. According to the resulting data, it is noticeable that the lipid content was higher in females than in males, both in wild and farmed sea urchin. For both sexes, lipid contents were higher in farmed than in wild specimens. Among identified saturated FAs, the most abundant were myristic (14:0) and palmitic (16:0). Among identified monounsaturated FA, palmitoleic acid (16:1*n*-7) and vacenic acid (18:1*n*-7) were in the top of the range, while docosahexaenoic acid (22:6*n*-3) had the highest figures among PUFAs. By comparing wild and farmed sea urchins, the farmed ones showed a significantly decrease in the relative proportion of total *n*-3 PUFAs, and a noticeable increase in monounsaturated FAs. No differences were found between males and females FA profiles for the two types of sea urchins.

P58. CASCADA QUIMIOENZIMÁTICA PARA LA PRODUCCIÓN DE L-AMINOÁCIDOS ÓPTICAMENTE PUROS

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Los aminoácidos son utilizados como aditivos alimentarios, en la fabricación de fármacos, cosméticos, pesticidas, curtidos sintéticos o reactivos quirales.[1]

El "Proceso de la Hidantoinasa" permite obtener D-aminoácidos ópticamente puros a partir de mezclas racémicas de hidantoínas monosustituidas en el carbono 5, de manera sencilla, barata y menos contaminante que los métodos de síntesis química y quimioenzimática.[2] Sin embargo, este sistema es muy poco eficiente para la producción de α-L-aminoácidos ópticamente puros.[3]

El objetivo de este trabajo es la producción de L-aminoácidos no naturales ópticamente puros a partir de D,L-hidantoínas-5-monosustituidas, mediante un sistema multienzimático derivado del "Proceso de la Hidantoinasa" formado por las enzimas D,L-hidantoinasa, hidantoína racemasa, L-carbamilasa y carbamil racemasa (Figura 1).

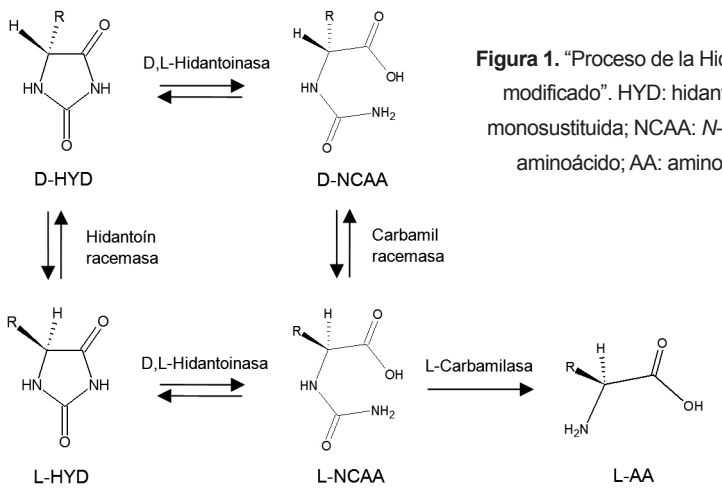

Figura 1. "Proceso de la Hidantoinasa modificado". HYD: hidantoína-5-monosustituida; NCAA: *N*-carbamil-aminoácido; AA: aminoácido.

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[1] Juaristi, E., López-Ruíz, H. *Curr. Med. Chem.* **1999**, 6: 983-1004.

[2] Clemente-Jiménez, J. M., Martínez-Rodríguez, S., Rodríguez-Vico, F., Las Heras-Vázquez, F. J. *Recent Patents Biotechnol.* **2008**, 2: 35-46.

[3] Wilms, B., Wiese, A., Syldatk C. Mattes, R., Altenbuchner, J. *J. Biotechnol.* **2001**, 86: 19-30.

P59. {[{(PTA)$_2$CpRu−μ-CN−RuCp(PTA)$_2$)}-μ-CoCl$_3$]}$_n$: A NEW MEMBER FOR THE FAMILY OF WATER SOLUBLE HETERO-METALLIC POLYMERIC COMPLEXES

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Ruthenium complexes have shown interesting properties useful in areas like catalysis, materials, photochemistry, biology, etc. Most of the ruthenium complexes are not water-soluble, which limits their use in water or systems with large concentration of water like biological systems. The main interest of the research team of the University of Almeria responsible of this communication is the synthesis and study of new water-soluble ruthenium complexes. One of the most interesting results obtained was the synthesis, characterization and study of the first example of a water soluble hetero-metallic organometallic polymer {[(PTA)$_2$CpRuDMSO]-AgCl$_2$}$_n$. This complex is air stable and display an interesting gel behavior in water solution. Other interesting example of water-soluble organometallic hetero-polymetallic compounds is the Ru-Ru-Au polymer {[{(PTA)$_2$CpRu−μ-CN−RuCp(PTA)$_2$}-μ-Au(CN)$_4$-]}$_n$ that is the first known coordination/organometallic thermogel in water. This complex additionally exhibits nice chemical, physical-chemical and biochemical properties.[1] As a consequence of the interesting properties of the two known examples of water-soluble hetero-polymetallic complexes the Almeria research team has devoted hard efforts to synthesize new examples of this family of complexes. In this communication we present the new water soluble, air stable Ru-Ru-Co polymer complex {[{(PTA)$_2$CpRu−μ-CN−RuCp(PTA)$_2$}-μ-CoCl$_3$-]}$_n$ **(Fig. 1)**.

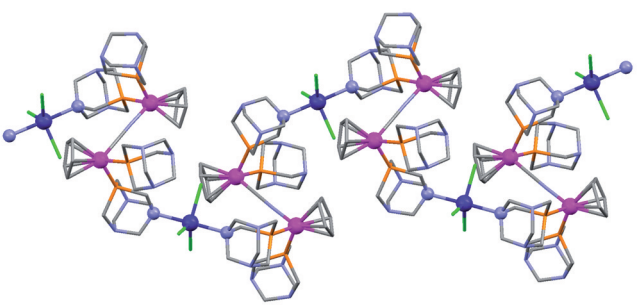

Figure 1. Crystalline structure of the water soluble air stable polymer {[(PTA)$_2$CpRu−CN−RuCp(PTA)$_2$]-CoCl$_3$-}$_n$

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[1] (a) Chaker Lidrissi, Antonio Romerosa, Mustapha Saoud, Manuel Serrano-Ruiz, Luca Gonsalvi, and Maurizio Peruzzini, *Angew. Chem. Int. Ed.* **2005**, *44*, 2568-2572. (b) M. Serrano-Ruiz, A. Romerosa, B. Sierra-Martin and A. Fernandez-Barbero, *Angew. Chem. Int. Ed.* **2008**, *47*, 1-6.

P60. BIOCHEMICAL AND MUTATIONAL STUDIES OF THE *BACILLUS CEREUS* CECT 5050T FORMAMIDASE SUPPORT THE EXISTENCE OF A C-E-E-K TETRAD IN SEVERAL MEMBERS OF THE NITRILASE SUPERFAMILY

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Formamidases (EC 3.5.1.49) are poorly characterized proteins. In spite of this scarce knowledge, ammonia has been described as playing a central role in the pathogenesis of human[1] pathogens such as *Helicobacter pylori*, for which formamidase has been shown to participate in the nitrogen metabolic pathway[2]. Sequence analysis has revealed that at least two different groups of formamidases are classified as EC 3.5.1.49: on the one hand, the derivatives of the FmdA-AmdA superfamily, which are the best studied to date, and on the other hand, the derivatives of *Helicobacter pylori* AmiF. Here we present the cloning, purification, and characterization of a recombinant formamidase from *Bacillus cereus* CECT 5050T (BceAmiF), the second member of the AmiF subfamily to be characterized, showing new features of the enzyme further supporting its relationship with aliphatic amidases. We also present homology modeling-based mutational studies confirming the importance of the Glu140 and Tyr191 residues in the enzymatic activities of the AmiF family. Moreover, we can conclude that a second glutamate residue is critical in several members of the nitrilase superfamily, meaning that what has consistently been identified for decades as a C-E-K triad[3,4] is in fact a C-E-E-K tetrad (Figure 1).

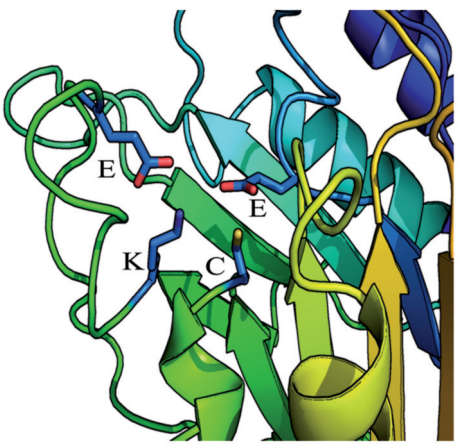

Figure 1. Modeled BceAmif showing the identified C-E-E-K tetrad in several members of the nitrilase family.

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[1] S. Skouloubris, A. Labigne, and H. De Reuse, *Mol. Microbiol.* **1997**, *25*, 989–998.

[2] C. Luiz-Borges, J. Alves-Parente, M. Santiago-Barbosa, J. Martins-Santana, S. Nair-Báo, M. Valle-De Sousa, C. M. De Almeida-Soares, *FEMS Yeast Res.* **2010**, *10*, 104–113.

[3] H. C. Pace, C. Brenner, *Genome Biol.* **2001**, 2, reviews0001.1–reviews0001.9.

P61. PURIFICATION OF AA-TRIACYLGLYCEROLS FROM ARASCO BY GRAVIMETRIC COLUMN CHROMATOGRAPHY

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Arachidonic acid (AA, 20:4*n*-6) is found in human breast milk and can directly affect infant growth. So the existence of AA in infant formulas is very important, especially for the purpose of supporting an adequate *n*-6/*n*-3 balance for the correct development of formula fed infants. Because of that, there is a high demand of AA from pharmaceutical and infant alimentary industries.

Purification of AA from different sources has been widely reported, and in most cases AA is obtained in the free fatty acid form or as methyl and ethyl esters. It seems that acyl groups in triacylglycerols (TAGs) show more metabolic activity and also human absorption is improved if acyl groups are supplied as TAGs.

The aim of this work was to purify an AA-enriched fraction of TAGs from commercial single cell oil named ARASCO® (38-44% AA content) by employing food-safe methodologies.
TAG profile of ARASCO was obtained by RP-HPLC and the fatty acid (FA) profile of each cluster was determined by GLC. Later, normal-phase chromatography was used to purify the fraction with the highest AA content by employing different mixtures of solvents (*n*-hexane, acetone and ethanol) with increasing polarity.

An AA-enriched fraction of TAGs with an AA content of 90.9% was obtained with a 56.3% purification yield (referred to the target peak).

This purification method allows the obtaining of a highly enriched AA fraction from a natural and renewable source, with the advantage of being available in the TAG form. It is also feasible to scale up and could be applied with alimentary purposes.

P62. FATTY ACID PROFILE OF MUSCLE LIPID OF CULTURED OF DUSKY GROUPER, EPINEPHELUS MARGINATUS (LOWE, 1834), (PISCES: SERRANIDAE)

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The dusky grouper, *Epinephelus marginatus* (Lowe, 1834), is very attractive for human consumption and a highly prized marine fish for the quality of their flesh. This makes it a very good candidate for intensive aquaculture because of their desirable taste, hardiness in a crowded environment and rapid growth efficient feed conversion, and high market value. Successful commercial farming of grouper will depend on several factors, mainly an acceptable muscle quality for consumers against free-living fish. One of the main quality indexes in farmed fish for human consumption are the lipid amount and fatty acid (FA) profile stored in muscle, the main edible fraction. Some *n*-3 and *n*-6 polyunsaturated FAs have been regarded as essential for human health due to the role they play in the prevention and treatment of a wide variety of disorders.

In this study, juvenile grouper (*Epinephelus marginatus*) with an average initial weight of 550 ± 0.100 g were introduced in a cage-based fish farm (Acuisleta S.L, La Isleta del Moro, Almería, Spain) and fed with a commercial diet with a total daily ration of 2% body-weight throughout the experiment. Fish were sampled (n=4) in autumn and summer and used for determining fillet composition.

Cultured grouper have low muscle lipid content in relation to other marine species, in autumn was of 0.61% in wet matter, increasing as fish grew in the farm to 1.47% in summer. Analytical data showed that the most abundant FAs were palmitic (PA, 16:0) among saturated FAs, oleic (OA, 18:1n-9) among monounsaturated FAs and docosahexaenoic (DHA, 22:6n-3) among polyunsaturated FAs. When considering *n* series, there was a clear predominance of *n*-3 that accounted almost twice the content of *n*-6.

In autumn, saturated and monounsaturated fatty acids (SFAs and MUFAs respectively) represented approximately 25 and 21% each of total fatty acid, reaching total polyunsaturated ones (PUFAs) a figure to 47%. Among these, the most abundant were those of the *n*-3 series. In summer, muscle content SFA increases to 28% and MUFA to 30% while total PUFA decreased to 38%.

8. Lista de principales autores